PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 21/02, 21/04, C12P 19/34, C12Q 1/68 — A1

(11) International Publication Number: WO 96/34875

(43) International Publication Date: 7 November 1996 (07.11.96)

(21) International Application Number: PCT/US96/06060

(22) International Filing Date: 1 May 1996 (01.05.96)

(30) Priority Data:

08/434,425	3 May 1995 (03.05.95)	US
08/437,667	3 May 1995 (03.05.95)	US
08/434,001	3 May 1995 (03.05.95)	US
08/433,585	3 May 1995 (03.05.95)	US

(60) Parent Applications or Grants
(63) Related by Continuation

US	08/434,425 (CIP)
Filed on	3 May 1995 (03.05.95)
US	08/437,667 (CIP)
Filed on	3 May 1995 (03.05.95)
US	08/434,001 (CIP)
Filed on	3 May 1995 (03.05.95)
US	08/433,585 (CIP)
Filed on	3 May 1995 (03.05.95)

(71) Applicants *(for all designated States except US)*: NEXSTAR PHARMACEUTICALS, INC. [US/US]; Suite 200, 2860 Wilderness Place, Boulder, CO 80301 (US). UNIVERSITY RESEARCH CORPORATION [US/US]; P.O. Box 1140, Boulder, CO 80306 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: JENSEN, Kirk, B. [US/US]; 1855 Athens Street #204, Boulder, CO 80302 (US). CHEN, Hang [CN/US]; 2050 Athens B3-F, Boulder, CO 80302 (US). MORRIS, Kevin, N. [US/AT]; Markt 24, A-5620 Schwarzach (AT). STEPHENS, Andrew [US/US]; 720 Leyden Street, Denver, CO 80220 (US). GOLD, Larry [US/US]; 1033 5th Street, Boulder, CO 80302 (US).

(74) Agents: SWANSON, Barry, J. et al.; Swanson & Bratschun, L.L.C., Suite 200, 8400 East Prentice Avenue, Englewood, CO 80111 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SYSTEMATIC EVOLUTION OF LIGANDS BY EXPONENTIAL ENRICHMENT: TISSUE SELEX

(57) Abstract

This invention discloses high-affinity oligonucleotide ligands to complex tissue targets, specifically nucleic acid ligands having the ability to bind to complex tissue targets, and the methods for obtaining such ligands. Tissue targets comprise cells, subcellular components, aggregates or cells, collections of cells, and higher ordered structures. Specifically, nucleic acid ligands to red blood cells ghosts, glioblastomas, and lymphomas are described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

Systematic Evolution of Ligands by Exponential Enrichment: TISSUE SELEX

This work was supported by grants from the United States Government funded through the National Institutes of Health. The U.S. Government has certain rights to this invention.

FIELD OF THE INVENTION

Described herein are methods for identifying and preparing nucleic acid ligands to tissues. Tissues are described herein as a collection of macromolecules in a heterogeneous environment. According to this definition, tissues encompass a single cell type, a collection of cell types, an aggregate of cells or an aggregate of macromolecules. The method utilized herein for identifying such nucleic acid ligands is called SELEX, an acronym for Systematic Evolution of Ligands by EXponential enrichment. Specifically disclosed herein are high-affinity nucleic acid ligands which bind to various tissues.

BACKGROUND OF THE INVENTION

A method for the in *vitro* evolution of nucleic acid molecules with highly specific binding to target molecules has been developed. This method, Systematic Evolution of Ligands by EXponential enrichment, termed SELEX, is described in United States Patent Application Serial No. 07/536,428, entitled "Systematic Evolution of Ligands by Exponential Enrichment", now abandoned, United States Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled "Nucleic Acid Ligands", United States Patent Application Serial No. 07/931,473, filed August 17, 1992, entitled "Nucleic Acid Ligands", now United States Patent No. 5,270,163 (see also PCT/US91/04078), each of which is herein specifically incorporated by reference. Each of these applications, collectively referred to herein as the SELEX Patent Applications, describes a fundamentally novel method for making a nucleic acid ligand to any desired target molecule.

The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of

binding affinity and selectivity. Starting from a mixture of nucleic acids, preferably comprising a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding, partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules, dissociating the nucleic acid-target complexes, amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity nucleic acid ligands to the target molecule.

The basic SELEX method has been modified to achieve a number of specific objectives. For example, United States Patent Application Serial No. 07/960,093, filed October 14, 1992, entitled "Method for Selecting Nucleic Acids on the Basis of Structure", describes the use of SELEX in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. United States Patent Application Serial No. 08/123,935, filed September 17, 1993, entitled "Photoselection of Nucleic Acid Ligands" describes a SELEX based method for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoinactivating a target molecule. United States Patent Application Serial No. 08/134,028, filed October 7, 1993, entitled "High-Affinity Nucleic Acid Ligands That Discriminate Between Theophylline and Caffeine", describes a method for identifying highly specific nucleic acid ligands able to discriminate between closely related molecules, termed Counter-SELEX. United States Patent Application Serial No. 08/143,564, filed October 25, 1993, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Solution SELEX", describes a SELEX-based method which achieves highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. United States Patent Application Serial No. 07/964,624, filed October 21, 1992, entitled "Methods of Producing Nucleic Acid Ligands" describes methods for obtaining improved nucleic acid ligands after SELEX has been performed. United States Patent Application Serial No. 08/400,440, filed March 8, 1995, entitled

"Systematic Evolution of Ligands by EXponential Enrichment: Chemi-SELEX", describes methods for covalently linking a ligand to its target.

The SELEX method encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX-identified nucleic acid ligands containing modified nucleotides are described in United States Patent Application Serial No. 08/117,991, filed September 8, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides", that describes oligonucleotides containing nucleotide derivatives chemically modified at the 5- and 2'-positions of pyrimidines. United States Patent Application Serial No. 08/134,028, *supra*, describes highly specific nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH_2), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). United States Patent Application Serial No. 08/264,029, filed June 22, 1994, entitled "Novel Method of Preparation of 2' Modified Pyrimidine by Intramolecular Nucleophilic Displacement", describes oligonucleotides containing various 2'-modified pyrimidines.

The SELEX method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in United States Patent Application Serial No. 08/284,063, filed August 2, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Chimeric SELEX" and United States Patent Application Serial No. 08/234,997, filed April 28, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Blended SELEX", respectively. These applications allow the combination of the broad array of shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable properties of other molecules. Each of the above described patent applications which describe modifications of the basic SELEX procedure are specifically incorporated by reference herein in their entirety.

Without question, the SELEX process is very powerful. However, to date the process has been successfully demonstrated primarily with pure, simple

demonstration that complex targets are also compatible with the SELEX process. Tissue SELEX allows one to obtain nucleic acid ligands to multiple targets simultaneously, and is analogous to performing individual SELEX experiments on all the discrete components of a particular tissue.

It is desirable to be able to obtain nucleic acid ligands to complex tissue targets for various reasons. First, tissue SELEX can be useful to obtain nucleic acid ligands when a distinct target is unknown but a general mode of action of the desired ligand is suggested. Second, tissue SELEX can be useful when nucleic acid ligands are desired based on functional results. Since whole tissues or cells can be used in the SELEX process, it is possible to select for nucleic acid ligands which produce a particular phenotype in the tissue or cell. Third, it can be desirable to obtain nucleic acid ligands to a complex tissue target when it is unclear which single target would be effective. It is also useful to obtain nucleic acid ligands to a complex tissue target if the purified target is unavailable or unstable in its purified form (i.e., a membrane protein). Tissue SELEX allows the potential generation of ligands to previously unknown targets, and may rival monoclonal antibodies as reagents for research, diagnostics and therapeutics.

BRIEF SUMMARY OF THE INVENTION

The present invention includes methods of identifying and producing nucleic acid ligands to complex targets such as tissues and the nucleic acid ligands so identified and produced. More particularly, nucleic acid ligands are provided that are capable of binding specifically to tissues which are macromolecules in a heterogeneous environment, such as whole cells or substructures thereof, aggregates of cells, collections of cells, aggregates of macromolecules and the like.

Further included in this invention is a method of identifying nucleic acid ligands to tissues comprising the steps of (a) preparing a candidate mixture of nucleic acids, (b) partitioning between members of said candidate mixture on the basis of affinity to tissue, and (c) amplifying the selected molecules to yield a mixture of nucleic acids enriched for nucleic acid sequences with a relatively higher affinity for

binding to tissue. Also included are nucleic acid ligands identified according to such method.

Another embodiment of the invention includes methods wherein a negative selection is performed in order to perfect the discrimination between subtle differences of similar tissue types. In this embodiment, the resulting ligands are specific not only for a particular tissue type, but can discriminate between subtly different tissues of the same type. For example, this method can discriminate between normal and abnormal tissue types, between induced and uninduced tissue types, etc.

In another embodiment of the invention, a method is provided for identifying previously unknown or uncharacterized epitopes which are components of a larger unknown macromolecule, on the tissue target. The ligands that are evolved by the present invention are capable of binding to previously unknown epitopes and the macromolecule which comprises the unknown epitope can then be identifed by standard methods. For example, ligands can be evolved to a previously unknown protein found in the context of a complex tissue target. The ligand of the invention can be used to purify the protein away from the tissue target by standard protein purification and identification methods. These standard methods include affinity purification, microsequencing and cDNA databank searches. In this aspect, the newly identified epitopes which are components of a larger unknown macromolecule, such as new or previously uncharacterized proteins, are provided by the invention. These new epitopes and the macromolecule of which they are a component will be useful as diagnostic and therapeutic agents as well as the ligands that helped identify them.

More specifically, the present invention includes nucleic acid ligands to red blood cell ghosts, human tumor cell lines, such as a T-cell lymphoblast cell line, CEMss, and an adherent cell line, the glioma U-251, including those ligands listed in Tables 1 and 2. Also included are nucleic acid ligands to the above-described tissues that are substantially homologous to any of the given ligands and that have substantially the same ability to bind the above-described tissues. Further included in this invention are nucleic acid ligands to the above-described tissues that have substantially the same structural form as the ligands presented herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the results of crosslinking a ligand to red blood cell ghosts [(c56t) (SEQ ID NO:4)] and nucleic acids of similar, but scrambled, sequences to red blood cell ghost membrane extracts. A distinct protein band is identified specifically by the ligand. Shown are a silver-stained 6% SDS gel and autoradiography of the same gel. Irradiations were performed with a hand-held transilluminator (254 nm) and samples were separated by gel electrophoesis under denaturing and reducing conditions. 1-0'irradiation c56t (SEQ ID NO:4); 2-5' irradiation c56t (SEQ ID NO:4); 3-0'irradiation scrambled oligo #1; 4-5'irradiation scrambled oligo #1; 5-0'irradiation scrambled oligo #2; 6-5'irradiation control oligo #2.

Figure 2 shows the photoaffinity crosslinking of the truncate ligand c56t to RBC ghosts. 10^7 ghosts were mixed with 1 nM of c56t and irradiated with a 254 nm hand-held transilluminator for 0 or 5 minutes. The irradiations were performed in the absence of cold competitor, with 10 μM cold c56t (as a specific competitor) or 10 μM cold c16t (as a non-specific competitor). The photoaffinity reactions demonstrate the high affinity and high specificity of the ligand-protein interaction. Shown are SDS-PAGE results under both reducing and non-reducing conditions (both conditions are denaturing). The doubling of the molecular weight of the crosslinked protein under non-reducing conditions suggests the target protein is a disulfide-linked hetero- or homo-dimer.

Figure 3 shows predicted secondary structures of six ligands which are the result of the RBC ghost SELEX. The six sequences are derived from the motif I (Figure 3A), II (Figure 3B) and III (Figure 3C) classes of sequences (two from each motif) are truncated to the smallest functional size, as based upon phylogenetic and computer folding algorithms. Base pairing within each molecule is predicted as based upon phylogenetic and computer folding algorithms. Notice that the two ligands from motif III share common primary and secondary structures, but are circularly permuted in relation to each other.

Figure 4 displays affinity photocrosslinking data for the motif I truncate c56t and the motif II truncate c16t. The nucleic acid ligands have been synthesized as shown in figure 1 with a six-carbon amino linker on the 5' end of each molecule. These 5' modified ligands were radiolabeled on their 3' end with alpha ^{32}P ddATP. The

amino linker was used to conjugate the ligands with the photocrosslinking reagent sulfo-HSAB. Approximately 5 nM ssDNA was mixed with 10 mM sulfo-HSAB in 200 mM triethylamine CO_2 (pH 9.5) and allowed to react 15 min. at room temperature and 15 min. at 37 degrees C. Approximately 10^7 ghosts were mixed with 10 nM of each ligand conjugate in a volume of 15 μl, incubated 30 min. at room temperature and irradiated for 100 pulses of a 308 nm excimer laser (175 mJ/pulse/cm^2). The reaction was then mixed with an equal volume of 2X reducing SDS loading buffer and run on a 4-12% gradient SDS polyacrylamide gel. The gel was run, fixed and dried. Radioactivity was detected by a Fuji phosphorimager. Shown are photocrosslinking reactions as described above for c56t and c16t, with two additional reactions for each ligand: one included the addition of 10 μM cold, unconjugated c56t, the other the same concentration of c16t. These "cross competition" reactions demonstrate the high affinity and specificity of the photoaffinity crosslinking method.

Figure 5 shows the results of three rounds of selection for sequences within the final round rbc ghost SELEX pool that are specific for four distinct proteins on the rbc ghost membrane. The final round SELEX pool (round 25) was amplified using a "sense-strand" primer synthesized with a 5' six carbon amino linker group. The PCR product was radiolabeled with 3,000 Ci/mmol, 1.3 μM (final) alpha ^{32}P dCTP ([cold dNTPS] = 100 μM (final)). The sense strand was purified using denaturing PAGE and eluted from the gel matrix and precipitated. The phenyl azide compound sulfo-HSAB was conjugated to the pool and the nucleic acid conjugate used for photoaffinity crosslinking with the rbc ghosts. 10^7 ghosts were irradiated with 10 nM pool conjugate in a volume of 15 μl and in the presence of 12 μM non-specific nucleic acid competitor (a 30 base random pool). The reaction was incubated for 30 min. at room temperature and irradiated for 100 pulses of a 308 nm excimer laser (175 mJ/pulse/cm^2). The reaction was then mixed with an equal volume of 2X reducing SDS loading buffer and run on a 4-12% gradient SDS polyacrylamide gel. The gel contents were electroblotted to a nitrocellulose filter, the filter washed in water and dried. Radioactivity was detected by a Fuji phosphorimager. DNA sequences which showed crosslinking to four proteins (termed proteins 5, 6, 7, and 8) varying in apparent molecular weight from 170-30 kDa were isolated by sectioning the

nitrocellulose filter and placing the appropriate filter slices directly into PCR reactions for sequence amplification. The sequences were amplified for approximately 22 rounds, the sense strand purified, and the DNA reamplified for another 22 rounds. The resulting DNA was again purified, conjugated to sulfo-HSAB and used for the next round of photoaffinity crosslinking. Figure 5 shows the photoaffinity crosslinking obtained after 3 rounds of the enrichment process described above.

DETAILED DESCRIPTION OF THE INVENTION

This application describes nucleic acid ligands to complex tissue targets identified generally according to the method known as the SELEX process. As stated earlier, the SELEX technology is described in detail, and incorporated herein by reference, in the SELEX Patent Applications. This method, referred to as the Tissue SELEX process, incorporates complex targets in contrast to the more simple targets previously used in the SELEX process. Certain terms used to describe the invention herein are defined as follows:

"SELEX" methodology refers to the combination of selection of nucleic acid ligands which interact with a target in a desirable manner, for example binding to a protein, with amplification of those selected nucleic acids as described in detail above and in the SELEX Patent Applications. Iterative cycling of the selection/amplification steps allows selection of one or a small number of nucleic acids which interact most strongly with the target from a pool which contains a very large number of nucleic acids. Cycling of the selection/amplification procedure is continued until a selected goal is achieved.

"Tissue SELEX" methodology applies the SELEX methodology to tissue targets. Tissue SELEX has several advantages. First, using Tissue SELEX one can obtain ligands to specific cell types in the absence of a defined understanding of the involved epitope. The epitope against which a ligand is evolved in usually a substructural component of a larger macromolecule The ligands found by this method could also be useful in identifying new proteins or other new macromolecules on the tissue target. The new proteins or other new macromolecules which comprise a newly identified epitope can be purified and characterized using standard procedures.

Second, ligands can be obtained to defined epitopes or macromolecules in the context of their physiologic cellular or membrane environment. Examples of various tissue targets can include a membrane protein on a whole cell, a plasma protein in plasma, a nuclear protein in the presence of whole nuclear extracts, etc. Third, it is possible to obtain ligands to tissues in a functionally altered phenotype, e.g., activated, migrating, etc. The ligands and the new macromolecules containing the ligand epitopes identified by this process may be useful as diagnostics or therapeutics. Fourth, Tissue SELEX is a powerful methodology which allows one to identify nucleic acid ligands that can mediate many different cell behaviors, such as apoptosis, anergy, differentiation, proliferation, etc., without prior knowledge of the identity of the specific tissue targets that control these changes. The sensitivity of the SELEX process may lead to the generation of oligonucleotides that recognize potentially every different epitope on the complex tissue target. Larger numbers of different sequence motifs are expected using the tissue SELEX process, as compared with simple-target SELEX, since it is believed that different motifs will recognize distinct epitopes on the complex tissue target. Some epitopes may lie within the same protein, but many will be directed to various proteins or other molecules on the tissue. Tissue SELEX can be done *in vivo* or *in vitro*.

Tissue SELEX allows one to work with a complete living "element" (a cell or bigger) that allow one to *phenotypically* screen for a target-ligand interaction that effects this "element." For example, one could screen an evolved, high affinity tissue SELEX pool using flow cytometry for sequences which bind a membrane protein and cause the cell to carry out a biochemical transformation which is measured by the flow instrument.

Tissue SELEX allows one to obtain nucleic acid ligands to multiple targets simultaneously. All independent binding sites on a very large macromolecular complex such as a tissue or cell should be potential targets for selection. In effect, this allows one to take a tissue and carry out numerous SELEX procedures on this tissue that is theoretically equivalent to individual SELEXes on all individual components of the particular tissue.

In one embodiment, a negative selection process (termed counter-SELEX) is employed to enhance the possibility that the ligands derived by tissue SELEX have precise specificity and affinity. In this embodiment, ligands are selected for a specific tissue and then a negative selection is done against a related tissue which does not have certain characteristics for which the ligand is desired. The negative selection can be done against a similar cell line or cell type, different cells, normal tissue, plasma or blood, a non-specific antibody or other available ligand. An example of this negative selection would be to first select using a tumor cell target (such as a malignant melanoma) and then counterselect the resulting nucleic acids against a similar cell type which is not tumorogenic (such as normal human melanocytes). Ligands that interact with both normal and neoplastic tissue will be removed by this negative selection and only those nucleic acid ligands that specifically bind the tumor cells will be identified (or retained). The resulting nucleic acid ligand would be specific for tumors. This technique will provide the ability to identify nucleic acid ligands that can discriminate between two closely related targets, i.e., between a cancerous cell and an untransformed cell of the same tissue type. The negative selection can also be done *in vivo*. Using this method one can not only generate ligands to specific targets on complex tissue surfaces, but also be able to recognize the differences between normal and abnormal tissue of a particular type.

"SELEX Target" or "Target" refers to any compound upon which a nucleic acid can act in a predetermined desirable manner. A SELEX target molecule can be a protein, peptide, nucleic acid, carbohydrate, lipid, polysaccharide, glycoprotein, hormone, receptor, antigen, antibody, virus, pathogen, toxic substance, substrate, metabolite, transition state analog, cofactor, inhibitor, drug, dye, nutrient, growth factor, cell, tissue, etc., without limitation. Virtually any chemical or biological effector would be a suitable SELEX target. Molecules of any size can serve as SELEX targets. A target can also be modified in certain ways to enhance the likelihood of an interaction between the target and the nucleic acid.

"Tissue target" or "Tissue" refers to a certain subset of the SELEX targets described above. According to this definition, tissues are macromolecules in a heterogeneous environment. As used herein, tissue refers to a single cell type, a

collection of cell types, an aggregate of cells, or an aggregate of macromolecules. This differs from simpler SELEX targets which are typically isolated soluble molecules, such as proteins. In the preferred embodiment, tissues are insoluble macromolecules which are orders of magnitude larger than simpler SELEX targets. Tissues are complex targets made up of numerous macromolecules, each macromolecule having numerous potential epitopes. The different macromolecules which comprise the numerous epitopes can be proteins, lipids, carbohydrates, etc., or combinations thereof. Tissues are generally a physical array of macromolecules that can be either fluid or rigid, both in terms of structure and composition. Extracellular matrix is an example of a more rigid tissue, both structurally and compositionally, while a membrane bilayer is more fluid in structure and composition. Tissues are generally not soluble and remain in solid phase, and thus partitioning can be accomplished relatively easily. Tissue includes, but is not limited to, an aggregate of cells usually of a particular kind together with their intercellular substance that form one of the structural materials commonly used to denote the general cellular fabric of a given organ, e.g., kidney tissue, brain tissue. The four general classes of tissues are epithelial tissue, connective tissue, nerve tissue, and muscle tissue.

Examples of tissues which fall within this definition include, but are not limited to, heterogeneous aggregates of macromolecules such as fibrin clots which are acellular; homogeneous or heterogeneous aggregates of cells; higher ordered structures containing cells which have a specific function, such as organs, tumors, lymph nodes, arteries, etc.; and individual cells. Tissues or cells can be in their natural environment, isolated, or in tissue culture. The tissue can be intact or modified. The modification can include numerous changes such as transformation, transfection, activation, and substructure isolation, e.g., cell membranes, cell nuclei, cell organelles, etc.

Sources of the tissue, cell or subcellular structures can be obtained from prokaryotes as well as eukaryotes. This includes human, animal, plant, bacterial, fungal and viral structures.

"Nucleic acid" means either DNA, RNA, single-stranded or double-stranded and any chemical modifications thereof. Modifications include, but

are not limited to, those which provide other chemical groups that incorporate additional charge, polarizability, hydrogen bonding, electrostatic interaction, and fluxionality to the individual nucleic acid bases or to the nucleic acid as a whole. Such modifications include, but are not limited to, modified bases such as 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at cytosine exocyclic amines, substitution of 5-bromo-uracil; backbone modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine and the like. Modifications can also include 3' and 5' modifications such as capping. Modifications that occur after each round of amplification are also compatible with this invention. Post-amplification modifications can be reversibly or irreversibly added after each round of amplification. Virtually any modification of the nucleic acid is contemplated by this invention.

"Nucleic acid test mixture" or "nucleic acid candidate mixture" is a mixture of nucleic acids of differing, randomized sequence. The source of a "nucleic acid test mixture" can be from naturally-occurring nucleic acids or fragments thereof, chemically synthesized nucleic acids, enzymatically synthesized nucleic acids or nucleic acids made by a combination of the foregoing techniques. In a preferred embodiment, each nucleic acid has fixed sequences surrounding a randomized region to facilitate the amplification process. The length of the randomized section of the nucleic acid is generally between 8 and 250 nucleotides, preferably between 8 and 60 nucleotides.

"Nucleic acid ligand" is a nucleic acid which has been isolated from the nucleic acid candidate mixture that acts on a target in a desirable manner. Examples of actions on a target in a desirable manner include, but are not limited to binding of the target, catalytically changing the target, reacting with the target in a way which modifies/alters the target or the functional activity of the target, covalently attaching to the target as in a suicide inhibitor, factilitating the reaction between the target and another molecule. In most, but not all, instances this desirable manner is binding to the target. In the most preferred embodiment, a nucleic acid ligand is a non-naturally occurring nucleic acid ligand having a specific binding affinity for a tissue target molecule. such target molecule being a three dimensional chemical structure other than

a polynucleotide that binds to said nucleic acid ligand through a mechanism which predominantly depends on Watson/Crick base pairing or triple helix binding, wherein said nucleic acid ligand is not a nucleic acid having the known physiological function of being bound by the target molecule. Nucleic acid ligand includes nucleic acid sequences that are substantially homologous to the nucleic acid ligands actually isolated by the Tissue SELEX procedures. By substantially homologous it is meant a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%. In the past it has been shown that the sequence homologies of various nucleic acid ligands to a specific target shows that sequences with little or no primary homology may have substantially the same ability to bind the target. For these reasons, this invention also includes nucleic acid ligands that have substantially the same ability to bind a target as the nucleic acid ligands identified by the Tissue SELEX process. Substantially the same ability to bind a target means that the affinity is within a few orders of magnitude of the affinity of the ligands described herein. It is well within the skill of those of ordinary skill in the art to determine whether a given sequence -- substantially homologous to those specifically described herein -- has substantially the same ability to bind a tissue target.

"Partitioning" means any process for separating nucleic acid ligands from the remainder of the unreacted nucleic acid candidate mixture. Partitioning can be accomplished by various methods known in the art. Filter binding, affinity chromatography, liquid-liquid partitioning, filtration, gel shift, density gradient centrifugation are all examples of suitable partitioning methods. Equilibrium partitioning methods can also be used as described in detail below. Since the tissue targets of the present invention are non-soluble, there are numerous simple partitioning methods which are well suited to this invention. The simple partitioning methods include any method for separating a solid from a liquid, such as, centrifugation with and without oils, membrane separations and simply washing the insoluble tissue target. The ligands can also be specifically eluted from the target with a specific antibody or ligand. The choice of partitioning method will depend on properties of the target and the nucleic acid and can be made according to principles and properties known to those of ordinary skill in the art.

"Amplifying" means any process or combination of process steps that increases the amount or number of copies of a molecule or class of molecules. In preferred embodiments, amplification occurs after members of the test mixture have been partitioned, and it is the facilitating nucleic acid associated with a desirable product that is amplified. For example, amplifying RNA molecules can be carried out by a sequence of three reactions: making cDNA copies of selected RNAs, using the polymerase chain reaction to increase the copy number of each cDNA, and transcribing the cDNA copies to obtain RNA molecules having the same sequences as the selected RNAs. Any reaction or combination of reactions known in the art can be used as appropriate, including direct DNA replication, direct RNA amplification and the like, as will be recognized by those skilled in the art. The amplification method should result in the proportions of the amplified mixture being essentially representative of the proportions of different sequences in the mixture prior to amplification. It is known that many modifications to nucleic acids are compatible with enzymatic amplification. Modifications that are not compatible with amplication can be made after each round of amplification, if necessary.

"Randomized" is a term used to describe a segment of a nucleic acid having, in principle, any possible sequence over a given length. Randomized sequences will be of various lengths, as desired, ranging from about eight to more than one hundred nucleotides. The chemical or enzymatic reactions by which random sequence segments are made may not yield mathematically random sequences due to unknown biases or nucleotide preferences that may exist. The term "randomized" is used instead of "random" to reflect the possibility of such deviations from non-ideality. In the techniques presently known, for example sequential chemical synthesis, large deviations are not known to occur. For short segments of 20 nucleotides or less, any minor bias that might exist would have negligible consequences. The longer the sequences of a single synthesis, the greater the effect of any bias.

A bias may be deliberately introduced into a randomized sequence, for example, by altering the molar ratios of precursor nucleoside (or deoxynucleoside) triphosphates in the synthesis reaction or the ratio of phosphoramidites in the chemical

synthesis. A deliberate bias may be desired, for example, to affect secondary structure, to introduce bias toward molecules known to have facilitating activity, to introduce certain structural characteristics, or based on preliminary results.

In its most basic form, the SELEX process may be defined by the following series of steps:

1) A candidate mixture of nucleic acids of differing sequence is prepared. The candidate mixture generally includes regions of fixed sequences (i.e., each of the members of the candidate mixture contains the same sequences in the same location) and regions of randomized sequences. The fixed sequence regions are selected either: (a) to assist in the amplification steps described below, (b) to mimic a sequence known to bind to the target, or (c) to enhance the concentration of a given structural arrangement of the nucleic acids in the candidate mixture. The randomized sequences can be totally randomized (i.e., the probability of finding a base at any position being one in four) or only partially randomized (e.g., the probability of finding a base at any location can be selected at any level between 0 and 100 percent).

2) The candidate mixture is contacted with the selected target under conditions favorable for binding between the target and members of the candidate mixture. Under these circumstances, the interaction between the target and the nucleic acids of the candidate mixture can be considered as forming nucleic acid-target pairs between the target and those nucleic acids having the strongest affinity for the target.

3) The nucleic acids with the highest affinity for the target are partitioned from those nucleic acids with lesser affinity to the target. Because only an extremely small number of sequences (and possibly only one molecule of nucleic acid) corresponding to the highest affinity nucleic acids exist in the candidate mixture, it is generally desirable to set the partitioning criteria so that a significant amount of the nucleic acids in the candidate mixture (approximately 5-50%) are retained during partitioning.

4) Those nucleic acids selected during partitioning as having the relatively higher affinity to the target are then amplified to create a new candidate mixture that is enriched in nucleic acids having a relatively higher affinity for the target.

5) By repeating the partitioning and amplifying steps above, the newly formed candidate mixture contains fewer and fewer unique sequences, and the average degree of affinity of the nucleic acids to the target will generally increase. Taken to its extreme, the SELEX process will yield a candidate mixture containing one or a small number of unique nucleic acids representing those nucleic acids from the original candidate mixture having the highest affinity to the target molecule.

The SELEX Patent Applications describe and elaborate on this process in great detail. Included are targets that can be used in the process; methods for partitioning nucleic acids within a candidate mixture; and methods for amplifying partitioned nucleic acids to generate an enriched candidate mixture. The SELEX Patent Applications also describe ligands obtained to a number of target species, including both protein targets where the protein is and is not a nucleic acid binding protein.

SELEX provides high affinity ligands of a target molecule. This represents a singular achievement that is unprecedented in the field of nucleic acids research. The present invention applies the SELEX procedure to more complicated tissue targets.

Negative selection (Counter-SELEX) is optionally employed before, during or after the Tissue SELEX process. The negative selection provides the ability to discriminate between closely related but different tissue types. For example, negative selection can be introduced to identify nucleic acid ligands that have a high specificity for a tumor cell but do not recognize the cognate normal tissue. Similarly, nucleic acid ligands can be identified which specifically recognize atherosclerotic arterial tissue but not normal arterial tissue. Nucleic acid ligands which recognize fibrin, but not fibrinogen can also be identified by this method. Additionally, nucleic acid ligands to a cell type which express a certain receptor can be counter-selected with a cell line engineered not to express the receptor (or other such macromolecule).

One of ordinary skill in the art will readily understand that various mechanisms can be employed to accomplish this negative selection. The following examples are provided mostly for illustrative purposes and are not meant in any way as limiting the procedures of negative selection. Negative selection or Counter-SELEX

methods were first described in United States Patent Application Serial No. 08/134,028, filed October 7, 1993, entitled "High-Affinity Nucleic Acid Ligands that Discriminate Between Theophylline and Caffeine", which is herein incorporated by reference. A particular implementation of negative selection is embodied using equilibrium partitioning. In this method, two cell lines or other tissue types are separated by a semi-permeable membrane (0.45- 0.90 μm pore size) in an equilibrium dialysis chamber; one cell line is the neoplastic target cell line, the other, the normal tissue used for the negative selection. The choice of cell or tissue type for the negative selection will be determined by the specific end results desired and will sometimes consist of a non-malignant cell line of the same tissue type as the neoplastic target. For other experiments, various normal cell types could be combined to create the negative epitope "sink." The random pool of nucleic acids is placed into the dialysis chamber (on the side of the normal cells; this avoids background from high avidity targets which are common to both the tumor and normal cells) and allowed to equilibrate between the two cell lines. Those nucleic acid sequences that remain bound to the target cell line or tissue at equilibrium are selectively recovered and amplified for the next round of SELEX.

This example of negative selection methodology is quite powerful. First, equilibrium dialysis negative selection allows the positive and negative selection to be carried out *simultaneously*. Second, the stringency of the negative selection can be varied through the alteration of the relative amounts of "positive" and "negative" cells placed on each side of the dialysis membrane. These two characteristics of equilibrium dialysis negative selection allow precise control over the evolution of nucleic acid ligands specific for the target cell or tissue type.

This same type of equilibrium partitioning negative selection can be carried out with adherent cell lines. In this embodiment, monolayers of target and negative cells or tissues are plated in different wells of a multi-welled plate. After adherence, media, along with an oligonucleotide pool, is added such that the wells are connected by the volume of cell media. After equilibration of the oligonucleotide pool, those sequences bound by the target cell line or tissue type would be isolated and amplified for the next round of SELEX.

The equilibrium negative selection strategies above offer a powerful way of generating nucleic acid ligands to tissue targets and especially tumor associated antigens (TAAs).

Additionally, there are several other negative selection methods, which could be classified as "post-SELEX screening procedures." The most simple of these procedures is the testing of individual nucleic acid ligands (those sequences generated by tissue SELEX and demonstrated to be high-affinity ligands for the tissue target) against normal tissue for cross-reactivity. However, this approach is a tedious and time-consuming process.

A more fruitful "post-SELEX" method is to perform a negative selection, for example using a normal tissue as the negative selection target, on a pool that has already been evolved from a SELEX against a desirable complex tissue target, for example a transformed cell line. This example would suggest the performance of two to three negative selections on a normal tissue using a late-round, highly evolved pool from a SELEX of a transformed cell line. The binding of certain sequences to the normal tissue would be used to subtract these sequences from the evolved pool. This method allows one to quickly eliminate from several hundred to several thousand nucleic acid sequences that show a high affinity for those targets common to both the normal and the transformed cell lines.

Another "post-SELEX" screening method is a variation of the photocrosslinking experiment described in Example two below. As an example, it is possible to synthetically incorporate a highly photoreactive nitrine group (which is also iodinatable) on the 5' end of a PCR primer used in the tissue SELEX protocols. Late-round pools from for example, a tumor cell line SELEX would be amplified with this photoactivatable (and ^{125}I-labeled) primer, and this sequence pool would then be irradiated in the presence of the tumor cell line, and in the presence of normal tissue. Membrane proteins would be isolated and solubilized for analysis on an SDS gel. One would expect to see many different protein epitopes tagged by specific oligonucleotide sequences, for both the tumor and the normal cell lines. A few tagged targets will be unique to the tumor cell line. Because the oligonucleotides have been photochemically linked to the protein targets in a manner which does not destroy the base sequence of

the oligonucleotide, it is possible to isolate a tumor-specific band from an SDS gel, and use PCR to recover a specific sequence motif that recognizes a particular tumor antigen. Thus, in one step, it will be possible to remove from a pool oligonucleotide sequences that recognize possibly hundreds of cell surface antigens, leaving one or a few families of sequences that bind specifically to a single tumor-specific antigen.

As described above, the Tissue SELEX methods can include the identification of macromolecules which comprise new epitopes on the tissue target. The nucleic acid ligand to the new epitope component of the macromolecule can be employed to purify, identify and characterize the macromolecule. The new macromolecule can be a previously unknown protein or peptide, lipid, carbohydrate, etc. Virtually any molecule that is part of the molecular make-up of a tissue can be identified by the Tissue SELEX process.

In order to fully exploit this aspect of the invention, it is important to develop strategies for the purification and identification of new macromolecules which comprise the new epitopes and to determine the roles these new macromolecular components of the tissue play in biological systems. The methods for purifying new macromolecules are well-known, especially in the art of protein purification. These standard purification methods include crosslinking, affinity chromatography, peptide microsequencing, Edman sequencing, mass spectrometry, and cDNA library searches.

The following discussion describes this process as it would be applied to the identification of a new tumor-associated antigen (TAA). For the purposes of this discussion, a TAA is a macromolecule that is expressed on a tumor cell, but not on a similar normal cell. A TAA may or may not be immunogenic. A TAA is merely one example of the kinds of macromolecules which can be identified by the Tissue SELEX process and simply used for illustrative purposes. However, it is readily apparent that this process can be extrapolated to any new macromolecule identified by the Tissue SELEX process.

As applied to TAAs, the identification of new TAAs by the Tissue SELEX process is composed of two main parts: one, developing strategies for the purification and identification of new TAAs, and two, the elucidation of the role these

tumor antigens play in cancer (i.e., determining the biological significance of each particular TAA in the development and progression of a particular cancer).

The steps of purification and identification of most of the TAAs should be straightforward and understood by one skilled in the art of protein purification. As with antibodies, SELEX provides a reagent –a high-affinity ligand specific for the tumor antigen– that is incredibly useful for the purification of the antigen from whole cells or other tissues. As a non-limiting example, most antigens will be amenable to some type of photo-affinity crosslinking as described in the RBC ghost SELEX experiments of Example 1 or in the negative selection strategies section above. Specific crosslinking of the TAA, using a photoactivatable oligonucleotide with a 3' biotin conjugate will allow one-pass purification of the TAA target using strepavidin coated beads. An alternative method to this purification strategy is to use a column-bound high-affinity nucleic acid ligand to affinity purify the TAA target from solubilized target cell membrane preparations.

There are many compelling reasons to believe that the method provided herein for identifying macromolecules that comprise new epitopes on tissues offers distinct advantages over traditional methods of new macromolecule discovery. Again, the following discussion will be directed to tumor-associated antigen discovery, but one will readily understand that it can be broadly extrapolated to all new macromolecule discovery.

As applied to tumor-associated antigens, one must fully consider that all that is known about tumor antigens has been derived from the immune system's reaction to particular antigens; science has depended on the particular restrictions of the immune system, and the system's repetoires to distinguish antigenic differences between neoplastic and normal tissue. It is entirely possible that other tumor antigens exist that are not subject to immune response. Some investigators have hypothesized that there may in fact be many antigenic differences between cancer and normal tissue, which are, unfortunately, not immunogenic .

The SELEX methodology provides an improved way to identify TAAs that avoids the restrictions posed by the immune system:

a. SELEX can actually provide a deeper search of TAAs than can the entire potential antibody repertoire of an organism– the size of the nucleic acid libraries used in SELEX is unrivaled by any biological system;

b. SELEX provides nucleic acid ligands to targets, including those which are not antigenic to the immune system because of tolerance. Many of the TAAs which have been identified are oncofetal– they are antigens expressed at some point during development or cell differentiation. As prior "self" antigens, they elicit no overt immune response because of earlier immune system tolerization. A SELEX-based search for TAAs avoids the circular nature of using the immune system as a means of identifying tumor antigens;

c. SELEX nucleic acid ligands have been shown to be exquisitely sensitive to target conformation. While most antibodies recognize conformational, or discontinuous eptitopes, antibody functional eptitopes are composed of only a few amino acids. The potential binding surface of an oligonucleotide ligand is much larger than that of an antibody variable region, and may provide greater conformational discrimination of large targets. Additionally, cross-reactivity for SELEX ligands is substantially less of a problem than for monoclonal antibodies. A considerable set of restrictions also controls T-cell mediated tumor responses. These immune system limitations provide important biological functions; however. they limit the immune system's power for TAA identification.

d. SELEX is possibly more sensitive to small quantities of antigen than the immune system. Although the immune system's threshold for reactivity has been estimated to be 200 copies/cell for an antigenic MHC-presented peptide, a B-cell antibody response (necessary for any antigen that is not a peptide– carbohydrates, lipids or conformational antigens) to a monovalent target requires antigen concentrations of about 100 mM. SELEX can generate ligands to TAA targets with a low representation on the cell surface;

e. SELEX provides a rapid and thorough method of TAA discovery. Screening of monoclonal antibodies to tissue sections, and purification and identification of MHC peptides are painstaking processes that set practical limits on

the depth and completeness of searches for TAAs. Tissue SELEX experiments take a much abbreviated length of time.

Nucleic acid ligands to tissue targets or the tissue epitopes identified by the method of the invention are useful as diagnostic reagents and as pharmaceuticals. The nucleic acid ligands are also useful for the identification of new macromolecules. The nucleic acid ligands are useful in any application that would be suitable for use of an antibody.

As diagnostic reagents, the ligands or tissue epitopes can be used in both *in vitro* diagnostics and *in vivo* imaging applications. The SELEX method generally, and the specific adaptations of the SELEX method taught and claimed herein specifically, are particularly suited for diagnostic appliations. SELEX identifies nucleic acid ligands that are able to bind targets with hgh affinity and with surprising specificity. These characteristics are, of course, the desired properties one skilled in the art would seek for a diagnostic ligand. Details regarding use of the ligands in diagnostic applications is well known to one of ordinary skill in the art. Nucleic acid ligands that bind specifically to pathological tissues such as tumors may have a role in imaging pathological conditions such as human tumor imaging and even therapeutic delivery of cytotoxic compounds or immune enhancing substances.

The nucleic acid ligands of the present invention may be routinely adapted for diagnostic purpses according to any number of techniques employed by those skilled in the art. Diagnostic agents need only be able to allow the user to identify the presence of a given target at a particular locale or concentration. Simply the ability to form binding pairs with the target may be sufficient to trigger a positive signal for diagnostic purposes. Those skilled in the art would also be able to adapt any nucleic acid ligand by procedures known in the art to incorporate a labelling tag in order to track the presence of a ligand. Such a tag could be used in a number of diagnostic procedures.

Specifically, oligonucleotide ligands with high specificity for particular tumor antigens could become as important as monoclonal antibodies for the detection, imaging, and surveillance of cancer. Modified nucleic acid ligands show nuclease resistance in plasma, and the use of 5' and 3' capping structures will provide stability

in animals that rivals that of monoclonal antibodies (and without the immunogenicity of animal-derived MAbs). Radionuclides, magnetic compounds, and the like can be conjugated to tumor-specific oligonucleotides for cancer imaging. SELEX tumor ligands can also be used to determine if these tumor antigens are sloughed off tumors, and are detectable in the plasma like PSA.

The nucleic acid ligands to tissue targets or newly identified macromolecules components of tissue are also useful as pharmaceuticals. Therapeutic uses include the treatment or prevention of diseases or medical conditions in human patients. Therapeutic uses also include veterinary applications. The ligands can bind to receptors and be useful as receptor antagonists. Conversely, under certain circumstances the ligands can bind to receptors and cause receptor capping and act as receptor agonists.

In order to produce nucleic acids desirable for use as a pharmaceutical, it is preferred that the nucleic acid ligand (1) binds to the target in a manner capable of achieving the desired effect on the target; (2) be as small as possible to obtain the desired effect; (3) be as stable as possible; and (4) be a specific ligand to the chosen target. In most situations, it is preferred that the nucleic acid ligand have the highest possible affinity to the target.

Standard formulations can be used for the nucleic acid ligands of the invention and are known to one of ordinary skill in the art.

The following examples provide a non-limiting description of the present invention. Example One describes obtaining ssDNA ligands to the complex tissue target red blood cell ghosts. The red blood cell ghost comprises a finite set of membrane-bound epitopes and is a non-living target which remained unchanged over the period of the selection. Ligands to RBC ghosts have numerous uses including, but not limited to, the ability to in vivo image extravascular blood as is desirable for head or retroperitoneal injuries or to extend the vascular half-life of other ligands that may be attached to the RBC ghost ligand. Example Two describes the identification of a macromolecule component on the RBC ghost using a ligand obtained in Example One. Example Three demonstrates that red blood cell ghost SELEX has produced high affinity and high specificity ligands to more than one macromolecular component of

the target cell membrance. Example Four describes the identification of and enrichment for high affinity nucleic acid ligands which bind individual components of a complex macromolecular target. Example Five describes obtaining ssDNA ligands to a glioblastoma cell line. High affinity and specificity nucleic acid ligands were isolated that may interact with tumor-associated (or tumor-specific) antigens, or mimic cytokines in their interactions with cell surface receptors causing cell morphology changes. Ligands to glioblastoma cell lines have numerous uses including, but limited to, in vivo imaging of glioblastomas, therapeutic localization of the ligand or other therapeutic agents that are attached thereto. Example Six describes ssDNA ligands to a human lymphoma cell line.

Example One

ssDNA Ligands to Red Blood Cell Ghosts

This example demonstrates the ability to obtain ssDNA ligands to the complex tissue target human red blood cell ghosts (RBC ghosts). Red blood cell ghosts are erythroid cells which have been lysed, purged of their cellular contents and preferentially resealed in a right-side-out manner (Steck et al. (1994) Biochemistry 10: 2617-2624). Red blood cell ghosts were the first complex tissue target on which in vitro selection was performed. The red blood cell ghost is one of the least complicated tissue targets and yet is still orders of magnitude more complex than the pure proteins or small molecules previously used for SELEX procedures. The red blood cell ghost comprises a finite set of membrane-bound epitopes and is a non-living target which remained unchanged over the period of the selection. Ligands to RBC ghosts have numerous uses including, but not limited to, the ability to in vivo image extravascular blood as is desirable for head or retroperitoneal injuries or to extend the vascular half-life of other ligands that may be attached to the RBC ghost ligand.

Briefly, the RBC ghost SELEX was carried out with single-stranded DNA for selection, using a 30-base randomized region. The single-stranded DNA pool was incubated with RBC ghosts, and the tighter-binding sequences were partitioned from the rest of the pool by filtering the reaction through nitrocellulose filters. 25 rounds of selection were carried out, using a decreasing concentration of

ghosts as the SELEX experiment progressed. The 25th round pool was cloned and sequenced according to standard procedures. Listed in Table 1 are the 66 sequences isolated from the 25th round pool (SEQ ID NOS: 5-70). Approximately 60% of these sequences can be classified into seven sequence-specific motifs, there is one class of pyrimidine-rich sequences (12%), and the other 19% are "orphans," showing no similarity to other sequences.

Binding behavior of round 0 and round 25 pools, and selected clones shows that the round 25 pool binds significantly better than the starting pool, and several of the motif 1 clones bind better than the round 25 pool. All sequences tested for binding so far show similar binding to whole red blood cells, so it is believed that the SELEX ligands have evolved to membrane targets on the extracellular side of the RBC ghosts.

A. MATERIALS AND METHODS

Red blood cell ghosts

Red blood cell ghosts are erythroid cells which have been lysed, purged of their cellular contents and preferentially resealed in a right-side-out manner (Steck et al. (1994) Biochemistry 10: 2617-2624). The concentration of protein in the preparation was measured with Coomassie brilliant blue G-250 (Bio-Rad).

Synthesis of initial pool of ssDNA

10 pmol of template with 30 random nucleotides flanked by fixed sequences complementary to the primers (SEQ ID NO: 1) was PCR amplified for 25 rounds in 10 mM Tris-HCl, pH 8.6, 50 mM KCl, 2.5 mM $MgCl_2$, 170 mg/ml BSA, 1 mM dNTPs, 0.5 units/ml *Taq* DNA polymerase and 5 mM each primer (5'-GGGAGCTCAGAATAAACGCTCAA-3' (SEQ ID NO: 2) and 5'-BBBGATCCGGGCCTCATGTCGAA-3'(SEQ ID NO: 3), where B=biotin). A similar reaction contained 1 pmol of template, 0.1 mM dCTP and 1.25 mM [α-^{32}P]dCTP (800 Ci/mmol) to produce internally labeled ssDNA for monitoring the binding affinity of the pool. Non-biotinylated, ssDNA was purified from the larger biotinylated strand by electrophoresis in 8% polyacrylamide gels containing urea.

The SELEX Protocol

40 pmol unlabeled ssDNA and a trace amount of radioactively labeled ssDNA were denatured by heating at 70°C for 5 min in 200 µl PBS (pH 7.3) and renatured at 0°C for 10 min. Pre-filtration of the DNA solution was used to counter-select sequences that might bind to nitrocellulose. After washing the filter with 300 µl PBS, the ssDNA molecules passed through the filter were divided into 50 µl aliquots. An equal volume of PBS containing various concentrations of RBC ghosts (0-1.72 mg/ml total protein) was added to each aliquot. The mixture was incubated for 20 min at room temperature then filtered through nitrocellulose. The filters were washed with 5 ml PBS and the amount of radioactively labeled ssDNA retained was measured by scintillation counting. The ssDNA was isolated from the filter that retained 5-10 times the radioactivity bound to the background control filter and was amplified by PCR for the next round of selection.

Nitrocellulose filter binding assays

The nitrocellulose filter partitioning method was used as described in SELEX Patent Applications to determine the affinity of nucleic acid ligands for RBC ghosts and for other proteins. Filter discs (nitrocellulose/cellulose acetate mixed matrix, 0.45 µm pore size, Millipore) were placed on a vacuum manifold and washed with 5 ml of TBSC buffer under vacuum. Reaction mixtures, containing ^{32}P labeled nucleic acid pools and RBC ghosts were incubated in TBSC for 5 min at 37 ° C, filtered, and then immediately washed with 5 ml TBSC. The filters were air-dried and counted in a Beckman liquid scintillation counter without fluor. Dissociation constants for single RBC ghost ligands were determined by Scatchard analysis (Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51:660-627; Robb, R.J., Munck, A., and Smith, K.A. (1985) J. Immunol. Methods 81:15-30), using constant ghost concentrations and varying the concentration of nucleic acid ligand. Scatchard analysis was performed using nitrocellulose partitioning of bound ligand from unbound ligand. For comparisons between random and evolved nucleic acid ligand pools, and for ligand/ligand comparisons, standard filter binding assays were used as described in the SELEX patent applications.

Cloning and nucleotide sequence determination

Individual DNA molecules were isolated from the round 25 pool by PCR amplification with primers that introduce *Bam*HI and *Hin*dIII restriction sites at the 5' and 3' ends of the DNA. Restriction digested PCR products were ligated into pUC18 and introduced into *E. coli* strain SURE (Stratagene) by electroporation. Plasmids were isolated and the nucleotide sequences in the inserted DNAs were determined by standard dideoxynucleotide methods. The sequences were searched for patterns in their primary sequences and in their possible secondary sequences both by inspection and with the aid of computer algorithms.

B. RESULTS OF THE SELEX PROCEDURE

Clones

As described in Section A, ssDNA with 30 randomized positions was used in SELEX with RBC ghosts as the target. The affinity of the ssDNA population for the membranes increased over twenty-five rounds of selection and amplification. The round 25 PCR products were cloned and the nucleotide sequences of 66 individuals were determined as shown in Table 1 (SEQ ID NO: 5-70). Eight clones contained one 8 and one 11 nucleotide consensus sequence separated by 3 to 14 bases (SEQ ID NOs: 5-12). This group of sequences has been termed motif I sequences. Several of these clones are likely to have arisen from a single progenetor sequence by PCR mutagenesis (ie. 20, 121 and 117). One of the clones (clone 25)(SEQ ID NO: 12) in this group may use a portion of the 5'-end fixed region to complete the consensus sequence. A region of this fixed sequence and the consensus sequence differ by only two nucleotides. Binding analysis of portions of the motif I sequences have defined the minimum binding sequence as the region containing no more than the 8-base and 11-base consensus sequences. Two synthetic truncate sequences have been made from the motif I sequences c56t (SEQ ID NO: 4) (from parent 56) and c20t (SEQ ID NO: 236) (from parent 20). The extremely high similarity between all the motif I sequences has prevented a phylogenetic analysis of the sequences and data on the secondary structure of this motif has not been obtained as shown in Figure 3.

Another group of 7 sequences (SEQ ID NOS: 22-25 and 35-37) contain an 18-base conserved primary sequence and share additional secondary structural elements. Computer folding algorithms and phylogenetic analysis predict a hairpin-bulge-stem structure for these sequences as shown in Figure 3. These sequences have been termed the motif II sequences. Two synthetic truncated ligands have been made for this sequence motif, c16t (SEQ ID NO: 237) (parent 16) and c79t (SEQ ID NO: 238) (parent 79).

An additional group of 10 sequences share a common region of 13 bases, surrounded by additional conserved secondary structural elements. Computer folding algorithms and phylogenetic analysis predict a stem-bulge-stem structure for this group of sequences, called the motif III sequences (SEQ ID NOS: 18-21; 28-30; 40-42) as shown in Figure 3. The similarity between the members of the motif III sequences becomes even more substantial at the secondary structure level, for the motif III ligands accomplish this structure in two different *circularly permuted* ways. Figure 3 illustrates this permutation for two motif III truncate ligands, c53t (SEQ ID NO: 240) (parent 53) and c111t (SEQ ID NO: 239) (parent 111).

Three more sequence motifs have been defined by sequence homology. Motif IV contains 5 members, motif V has 5 members, and motif VI 2 members as shown in Table 1. The possible secondary structures for these sets of ligands has not yet been determined.

Twenty of the sequences show no large sequence homology to other sequences and are termed orphans. While several identical clones lie within this group, these clones most likely arose from a single progenitor sequence and do not represent another "motif."

The final group of sequences showed extremely high pyrimidine content (77-90 %), and no common secondary structure has been proposed.

Affinities

The binding behavior of round 0 and round 25 pools, and a selected number of round 25 clones have been tested. The round 25 pool binds approximately 10-fold better than the starting pool, and several of the motif I clones bind 100-fold

better than the round 0 pool. All sequences tested for binding show similar binding to whole red blood cells, and therefore the inventors hereof believe that ligands have been selected to membrane targets on the extracellular side of the RBC ghosts.

A synthetic twenty-two nucleotide truncate of clone 56 (c56t)(SEQ ID NO: 4) that contains only the consensus sequences with four intervening nucleotides retained most of the binding affinity exhibited by the entire ssDNA sequence. A Scatchard plot analysis of c56t measured 1600 binding sites per cell, and a calculated dissociation constant of 4 nM for the target presented on the RBC ghosts. Truncate ligands from motifs II and III have not yet been analyzed for binding to the ghosts, but the photoaffinity studies of these ligands shown in Examples 3 and 4 indicates that their dissociation constants are as good or better than c56t. The pyrimidine-rich clones had affinities that were higher than the round 25 pool but lower than the consensus clones.

Example Two

Identification of Macromolecule Component on RBC Ghost

In order to confirm that the c56t ligand (SEQ ID NO: 4) recognizes a single, distinct target on RBC ghosts, a series of short-wavelength UV crosslinking experiments were done in an effort to photochemically link the c56t ligand to its membrane target through thymidine crosslinking. As controls, two 22-base DNA oligonucleotides of the same base composition, but scrambled in sequence were also crosslinked to the RBC ghost target. Briefly, the target recognized by c56t was identified by short wavelength (254 nm) UV crosslinking experiments. 5' ^{32}P end labelled truncate ligand c56t, and two control oligonucleotides of the same length and base composition (but with the primary sequences scrambled using a "shuffling" computer algorithm), were irradiated in the presence of RBC ghosts. The ghost membrane proteins were fractionated using denaturing SDS gel electrophoresis, and the presense of crosslinked ligand detected by autoradiography of the dried gel. The results are shown in Figure 1. Autoradiography indicated a single specific crosslinked product for c56t (all three oligos show slight crosslinking to two other RBC ghost proteins). The c56t ligand, but not the two controls, selectively labels an RBC ghost

membrane protein with an apparent molecular weight of 105 kDa. Silver staining of this protein target indicates that it is not an abundant protein.

A similar short wavelength photoaffinity crosslinking experiment was performed using both specific and non-specific nucleic acid competitor in the photocrosslinking reaction (Figure 3). The addition of a 10^3 molar excess cold c56t in the reaction abolished crosslinking to the 105 kDa ghost component. However, the addition of a 10^3 molar excess of cold motif II sequence c16t did not affect the crosslinking of c56t. This "cross competition" experiment demonstrates the incredible affinity and specificity of the truncate ligand c56t with its protein target.

Additionally, the product of the photoaffinity crosslinking reaction was examined under both reducing and non-reducing SDS-PAGE as shown in Figure 2. Under reducing conditions, the crosslinked protein runs with an apparent molecular weight of 105 kDa. Under non-reducing conditions, the crosslinked protein migrates at about 210 kDa, and suggests that the crosslinked protein is present on the ghost membrane as a disulfide-linked hetero- or homo-dimer. At present, only two human CD antigens that are disulfide bonded homodimers with monomer molecular weights within the range of 90-110 kDa are known, and only one is present on red blood cells and its direct progenitors. This antigen is the transferrin receptor (with a monomer molecular weight of 95 kDa). A definitive demonstration of the identity of the protein crosslinked by c56t is under investigation.

Example Three

Red Blood Cell Ghost SELEX has produced high affinity and high specificity ligands to more than one macromolecular component of the target cell membrane

A key assumption of tissue SELEX is that nucleic acid selection of collections of large macromolecular structures should result in the generation of high affinity ligands to all independent binding sites on these structures. Since cells or tissues are many magnitudes of order larger than a purified protein target, the number of these independent binding sites should be large. In brief, this theory predicts that selection of multiple targets produces ligands with multiple binding specificities.

Thus the selection of red blood cell ghosts should result in the evolution of high affinity nucleic acid ligands to more than one, and potentially all protein targets present on the membrane surface. In an effort to provide definitive proof of this hypothesis, truncate ligands from the first two red blood cell ghost sequence classes (motif I and II; see Figure 3) were affinity photocrosslinked to the ghost membranes. Truncates c56t (motif I) (SEQ ID NO: 4) and c16t (motif II) (SEQ ID NO: 237) were made synthetically, with the addition of a primary aliphatic amine (with a six carbon spacer group) on the 5' end of each molecule. This amino group was used to conjugate the truncate ligands to the phenyl azide photoreactive molecule sulfo-HSAB (N-hydroxysulfo-succinimidyl 4-azidobenzoate, Pierce Chemical Company). Additionally, these molecules were radiolabeled on their 3' end using alpha 32^P ddATP. The truncate ligand conjugates were mixed with ghosts and photocrosslinking carried out using a 308 nm excimer laser as shown in Figure 4. To demonstrate high affinity and specificity, the photoreactive truncates were irradiated with the ghosts in the presence of cognate or non-cognate unradiolabeled, unconjugated truncate.

The motif I truncate ligand c56t specifically labels a dimer protein band of apparent molecular weight of 105 kDa, the identical protein band labeled by this truncate using short wavelength UV photocrosslinking. This photoaffinity crosslinking can be prevented by the addition of 10^4 molar excess of "cold" c56t, but not by the addition of 10^4 molar excess of cold c16t. Similarly, the motif II truncate specifically labels a protein of apparent molecular weight of 40 kDa. This crosslink can be prevented by the addition of cold c16t but not by cold c56t. Thus, it is clear the red blood cell ghost SELEX has produced high affinity and high specificity ligands to more than one macromolecular component of the target cell membrane.

This photoaffinity analysis has now been carried out for all truncate ligands shown in Figure 3. The motif I truncate c20t (SEQ ID NO: 236) specifically labels the same protein dimer band as the motif I truncate c56t, and the motif II truncate c79t (SEQ ID NO: 238) labels the same 40 kDa protein band at the motif II truncate c16t. The two motif III truncate ligands c53t (SEQ ID NO: 240) and c111t (SEQ ID NO: 239) specifically label a group of three proteins ranging in molecular

weight from 42-55 kDa, and presumably these proteins are physically associated as a protein complex on the ghost membranes. This consistent pattern of identical photoaffinity crosslinking behavior *within* sequence motifs, and different protein bands crosslinking *among* motifs is very strong proof of the fundamental hypothesis of tissue SELEX– multiple targets result in ligands with multiple specificities.

Example Four

Identification of and enrichment for high affinity nucleic acid ligands which bind individual components of a complex macromolecular target

After the generation by tissue SELEX of high affinity ligands to many targets within a complex mixture, it is desirable to be able to screen this large pool of sequences for those nucleic acid molecules which recognize a particular, discrete target within the complex mixture. A method for this procedure has been developed for the red blood cell ghost SELEX which has been termed "pool deconvolution." The pool of sequences from the final round of the RBC ghost SELEX (round 25) was amplified using internal radiolabel and a "sense strand" PCR primer which carried the same primary amine, six carbon spacer described in Example Three at its 5' end. Thus, every sequence in the purified ssDNA pool contained this primary amino group at their 5' end. The pool of sequences was conjugated to the phenyl azide compound sulfo-HSAB, purified, and incubated with the RBC ghosts in the presence of 10^3 molar excess of non-specific nucleic acid competitor. The mixture was irradiated using a 308 nm excimer laser and the crosslinked products separated by SDS-PAGE.

The crosslinking pattern of the final round pool is shown in Figure 5. One can clearly see that many different proteins present in the ghost membrane have been specifically photolabeled by the pool sequences. The SDS-PAGE separated products were electroblotted to a nitrocellulose filter, and sections of the filter which corresponded to four different crosslinked proteins were excised and placed in PCR reactions for amplification of the pool sequences which crosslinked to the particular protein selected. This "deconvolution SELEX" was carried out for three rounds, and the results of the selections are shown in Figure 5. Lanes numbered 5, 6, 7, and 8 correspond to the four selected protein bands as indicated on the round 25 lane. The

three rounds of selection has produced excellent enhancement for sequences which can specifically photocrosslink to selected ghost membrane proteins. The pools used to produce lanes 5 and 8 are both close to becoming completely specific for the selected proteins. The stringency of further selections will be increased by using high concentrations of non-specific competitor nucleic acid and by competing a particular pool (such as that for band 5) with cold, non-conjugated fractions of the remaining three pools. Such a scheme should allow the selective removal of sequences that are common to two or more pools. For example, competing the photocrosslinking of the pool for band 5 with cold material from the band 6, 7, and 8 pools should eliminate the common crosslinking between the band 5 pool and the other pools. When the selection is completed, the isolated DNA for a particular protein band can be readily sequenced by standard methods, allowing one to correlate particular nucleic acid sequences with high affinity binding to a discrete protein. This deconvolution technique is a powerful method for screening high affinity tissue SELEX pools for sequences which bind a particular target of interest.

Example Five
ssDNA Ligands to Glioblastoma U251 Cell Line

This example demonstrates the ability to obtain ssDNA ligands to the complex tissue target glioblastoma cell line U251, which is derived from human brain tumor (Hum. Hered. (1971) 21:238). High affinity and specificity nucleic acid ligands were isolated that may interact with tumor-associated (or tumor-specific) antigens, or mimic cytokines in their interactions with cell surface receptors causing cell morphology changes. Many of the protocols used in this example are outlined in Example One or are slightly varied as described below. Ligands to glioblastoma cell lines have numerous uses including, but not limited to, in vivo imaging of glioblastomas, therapeutic localization of the ligand or other therapeutic agents that are attached thereto.

In this tissue SELEX example, a fluorescent-labeled single-stranded DNA library with 34 nucleotide randomized region was used (SEQ ID NO: 71). The fluorescent-labeled ssDNA was purified by denaturing polyacrylamide gel. The sequences of primers and template are as follows:

5'-primer: 5'-F-GCCTGTTGTGAGCCTCCT-3' (F: fluorescein) (SEQ ID NO: 72)

3'-primer: 5'-GGGAGACAAGAATAAGCG-3' (SEQ ID NO: 73)

template:

55'-GCCTGTTGTGAGCCTCCT-N34-CGCTTATTCTTGTCTCCC-3' (SEQ ID NO: 71)

Briefly, the SELEX procedure was as follows. One to 10 million glioblastoma cell line U251 cells were washed twice in a culture flask with 20 mL cold RPMI-1640 serum-free medium at 4°C. 50-100 picomoles of ssDNA in 100 μL PBS buffer was heated at 90°C for 5 minutes and put on ice for 5 minutes. The ssDNA pool was added to the cell culture in 20 mL RPMI-1640 medium along with 20-40 fold excess sonicated sperm DNA and yeast tRNA (molar ratio 1:1). The solution was incubated at 4°C for 20 minutes with gentle shaking. The cells were washed twice with 20 mL cold RPMI-1640 medium to remove the free oligonucleotides. The cells were trypsinized with 1 mL of 0.25% trypsin. The solution that contains cells and oligonucleotides was collected to a 2 mL tube, boiling at 95°C for 5 minutes, followed by phenol extraction and ethanol precipitation. The recovered ssDNA was used for PCR amplification. Through 20 rounds of selection, the binding affinity of the final pool was significantly increased comparing with that of the starting material. The affinity increase was revealed by Scatchard graph. The round-20 pool was cloned into pUC18 vector by DUG cloning as described by Rashtchain *et al.* (Anal. Biochem. (1992) 206:91). About 158 sequences were obtained, which can be grouped into 22 subfamilies and are shown in Table 2 (SEQ ID NOs: 74-232).

Example Six
ssDNA Ligands to Human Lymphoma Cell Line

This example demonstrates the ability to obtain ssDNA ligands to the complex tissue target human lymphoma cell line CEMss, which is a CD4 positive cell line (Foley et al., Cancer (1965) 18:522). Many of the protocols used

in this example are outlined in Example One or are slightly varied as described below.

In this tissue SELEX example, fluorescein labeled single-stranded DNA molecules were used for the generating of combinatorial library. The fluorescein-labeling allows for image of oligonucleotides binding to the cell surface and for the purpose of flow cytometry. The sequences of primers and templates are as follows:

5'-primer: 5'-F*-GCCTGTTGTGAGCCTCCT-3' (F*=fluorescein) (SEQ ID NO: 233)

3'-primer: 5'-GGGAGACAAGAATAAGCG-3' (SEQ ID NO: 234)

template:

5'-GCCTGTTGTGAGCCTCCT---N_{34}---CGCTTATTCTTGTCTCCC-3'

(SEQ ID NO: 235)

Briefly, the SELEX procedure was as follows. The target cell line was the human lymphoma cell line CEMss, which is CD4 positive. $5X10^6$ cells were washed twice with 10 mL of cold PBS buffer in a 15 mL conical tube. The cells were resuspended with 1 mL PBS and stored on ice. 50-100 picomoles of fluorescein-labeled (and ^{32}P-internally-labeled by PCR) single-stranded DNA (SEQ ID NO: 235) in 100 μL PBS was heat denatured at 90°C for 5 minutes, and was kept on ice for 5 minutes. Incubate the single-stranded DNA together with 20-50 fold excess competitor yeast tRNA and sonicated denatured sperm DNA (ratio: 1 to 1), with cells at room temperature for 20 minutes with gentle shaking. Load the reaction solution on top of 0.5 mL of binding oil (84% silicon oil and 16% paraffin oil), spin at top speed for 15 seconds, immediately freeze in dry ice/ethanol. Cut the bottom tip of the tube off and put the tip in a 2 mL tube, add 100 μL water, 100 μL 7 M urea, and 400 μL phenol, shake and boil for 5 minutes. Count the cpm, then shake for another 20 minutes, spin at top speed for 10 minutes, transfer the top phase to a new tube and ethanol precipitate. The recovered DNA was PCR amplified and purified on a denaturing gel. The fluorescein-labeled strand migrates slower. The recovered ssDNA was used for next round of SELEX.

The improvement of binding affinity was determined by binding assay. The reaction condition was as described above, with the exception that the reaction volume is 100 μL, without the addition of competitor. After 12 rounds of selection the binding affinity increased compared to the zero round pool. The complexity of the round 12 pool is still relatively high and rounds will continue until the resulting complexity of the pool has somewhat decreased.

Table 1

RBC Ghost SELEX

SEQ ID NO:		
	Starting Pool	
1		gggagctcagaataaacgctcaa[30N]ttcgacatgaggcccggatc
	Motif I	
5	47/113	gggagctcagaataaacgctcaaCTCAGTGGT----AGGTAACGGTTCAAGACGGGAttcgacatgaggcccggatc
6	56	gggagctcagaataaacgctcaaCTCAGTGGT----AGGTAACGGTTATATCCGGAAttcgacatgaggcccggatc
7	8	gggagctcagaataaacgctcaaAACTCAGTATA----AGGTAACGGTTCCAACCCAGAttcgacatgaggcccggatc
8	20/121	gggagctcagaataaacgctcaaACTCAGTAATGCCAAGGTAACGGTTCCCTTttcgacatgaggcccggatc
9	117	gggagctcagaataaacgctcaaACTCAGTAATGCT-AGGTAACGGTTCCCTTttcgacatgaggcccggatc
10	15	gggagctcagaataaacgctcaaACTCAGTAATGCACCAGTAACGGTTACATCttcgacatgaggcccggatc
11	85/104	gggagctcagaataaacgctcaaCTCAGTAGCA---AGGTAACGGTTCAGATCCACttcgacatgaggcccggatc
12	25	agctcagaataaacgctcaa- GTCATAACGGTTAGCCAGAGGACCGTGCCttcgacatgaggcccggatc
4	c56t	aaCTCAGTGGT----AGGTAACGGTT
236	c20t	aACTCAGTAATGCCAAGGTAACGGTT
	Motif II	
35	16	gggagctcagaataaacgctcaaACGAATCGCATTGCCCAA-CGTTGCCC-AAGAttcgacatgaggcccggatc
36	43	gggagctcagaataaacgctcaaCCGAATCGCATTGCCCAA-CGTTGCCC-AAGAttcgacatgaggcccggatc
37	78	gggagctcagaataaacgctcaaTGTCGGATAAGTCGCCCAA-CGTTGCCC--ATTttcgacatgaggcccggatc
22	79	gggagctcagaataaacgctcaaTAG-TTGCCCA-CCGTTGTCC-AATTGATCGTAttcgacatgaggcccggatc
23	101	gggagctcagaataaacgctcaaTGG-TTGCCCAT-CGTTGTCC-AATTGATCGTTttcgacatgaggcccggatc
24	46	gggagctcagaataaacgctcaaT-G-TTGCCCATTCGTCGTCC-AAGTGAACGTttcgacatgaggcccggatc
25	66	gggagctcagaataaacgctcaaTGAATTGCCCAA-CGTCGCCCGAA-TGATGCGttcgacatgaggcccggatc
237	c16t	CGAATCGCATTGCCCAA-CGTTGCCC-AAGAttcg
238	c79t	cgctcaaTAG-TTGCCCA-CCGTTGTCC-AATTGAGCG

Table 1 (cont.)

	Motif	III
18	11	gggagctcagaataaacgctcaaGTGGAGTCGACACGCTGTGACCTTTG-GCATttcgacatgaggcccggatc
19	119	gggagctcagaataaacgctcaaGTG-AGTCGACACGCCGCGACCTTTG-GTATttcgacatgaggcccggatc
20	111	gggagctcagaataaacgctcaaGTG-CGTCGAGGCATTGCAACCTTTG-GTCTttcgacatgaggcccggatc
21	18	gggagctcagaataaacgctcaaTAGACCGTCGATGC-TTGCAACTTTAC-GTATttcgacatgaggcccggatc
28	22	gggagctcagaataaacgctcaaTGAGAGGGGCAACC-TTGAGTCTTTCATGCCttcgacatgaggcccggatc
29	53	gggagctcagaataaacgctcaaAGCAGCGGGCAACC-TTGAGTATTTCATGCttcgacatgaggcccggatc
30	132	gggagctcagaataaacgctcaaACCCGGGCAACCGTTCGGTCTTTCAGTCTttcgacatgaggcccggatc
40	7	gggagctcagaataaacgctcaaCATCTGGATGTTCAACCTTCTGGTCTTGCGttcgacatgaggcccggatc
41	21	gggagctcagaataaacgctcaaCTACCCGGTTGAACCTTC-GCTCTTGCGTAGttcgacatgaggcccggatc
42	38	gggagctcagaataaacgctcaaTGCTCCCCGAAACCCT-ATTTCTTGCTGCTAttcgacatgaggcccggatc
239	c111t	GTCGAGGCATTGCAACCTTTG-GTCTttcgac
240	c53t	GGGCAACC-TTGAGTATTTCATGCttcgacatgaggcccg
	Motif	IV
31	42	gggagctcagaataaacgctcaaCATCG-TTGACACCCTCGT---GTGCTTCAGGTAttcgacatgaggcccggatc
32	57	gggagctcagaataaacgctcaaCATCGCTTGACA-GCTGTG---CTGCTTCAGTTTttcgacatgaggcccggatc
33	73	gggagctcagaataaacgctcaaGGGTGATCGAAGCCTAGGT---GAGCTTGAGCCttcgacatgaggcccggatc
34	105	gggagctcagaataaacgctcaaGGGTGTCCGA-GCATCCGT----AGCTTGAGTCGTttcgacatgaggcccggatc
55	17	gggagctcagaataaacgctcaaAGAGGAGTC-TTGCTG--TCCGTACACAGCTTAttcgacatgaggcccggatc
	Motif	V
26	26	gggagctcagaataaacgctcaaAGGCGGTGT--------TACTTCTCACGAATTGAGGAAGttcgacatgaggcccggatc
27	39	gggagctcagaataaacgctcaaAG-CGTTGT--------TACTTCTCACGAATTGAGGAAGttcgacatgaggcccggatc
54	13	gggagctcagaataaacgctcaaGGAGCGCGATACGTTTACTTCTGATCATGttcgacatgaggcccggatc
65	108	gggagctcagaataaacgctcaaTAGGCCGGGTGAGC---TACTTCTAGTAGGGTGttcgacatgaggcccggatc
53	6	gggagctcagaataaacgctcaaTAGGGGTAGGGCGCAA-TA-TTCACCGGGCCttcgacatgaggcccggatc
	Motif	VI
17	5	gggagctcagaataaacgctcaaGGTTGTCGACGCATTATAGCGACATCGTCTttcgacatgaggcccggatc
16	58	gggagctcagaataaacgctcaaGGCGTGTCGATGTGGAATCACAAC-CTGTCTttcgacatgaggcccggatc

Table 1 (cont.)

	Orphans		
13	37	gggagctcagaataaacgctcaaCAGGTCGATCGAGTCAGGTAGGCGCCGAGAttcgacatgaggcccggatc	
14	51	gggagctcagaataaacgctcaaGAGGTCGATCGAGTCAGGTAGGCGCCGAGAttcgacatgaggcccggatc	
15	131	gggagctcagaataaacgctcaaCAGGTCGATTGAGTCAGGTAGGCGCCGAGAttcgacatgaggcccggatc	
38	81	gggagctcagaataaacgctcaaGTGGAGCGATTCGCGAAAATCGACTTGCATttcgacatgaggcccggatc	
39	116	gggagctcagaataaacgctcaaCTGGAGCGATTCGG-AAAATCGACTTGCATttcgacatgaggcccggatc	
52	4	gggagctcagaataaacgctcaaGTGGCCTCAAACTGCTAGGAGTAAACATGTttcgacatgaggcccggatc	
56	24	gggagctcagaataaacgctcaaTCCCTTGAACCATCGGTCTTGCGTTCCATGttcgacatgaggcccggatc	
67	110	gggagctcagaataaacgctcaaTCCGGAAAGCAACGCATACTTCGCATGTCGttcgacatgaggcccggatc	
63	84	gggagctcagaataaacgctcaaGGGCAATACACAACACTCTACCTCACCTCAttcgacatgaggcccggatc	
66	109	gggagctcagaataaacgctcaaGTTGTGATCCATTAGCGGCACCGCCTCCAttcgacatgaggcccggatc	
59	48	gggagctcagaataaacgctcaaGACAGCGTGATTCCTCCGCTCTGCTGCTATttcgacatgaggcccggatc	
68	123	gggagctcagaataaacgctcaaGTGAGCGTACCGGAGTGTGTTACCAATTAttcgacatgaggcccggatc	
57	28	gggagctcagaataaacgctcaaACAAGAGGGTCTTGCCGCACCATTCGGCTAttcgacatgaggcccggatc	
58	44	gggagctcagaataaacgctcaaACGAGTTACAGCCACCCATGCTGTCGGTGAttcgacatgaggcccggatc	
60	60	gggagctcagaataaacgctcaaCGGGACCTTGAGTATTCCTCATTATCGTTCTtcgacatgaggcccggatc	
62	70	gggagctcagaataaacgctcaaAGCCGAATTAGTAGCGTATAGCGTGTTGTGttcgacatgaggcccggatc	
64	107	gggagctcagaataaacgctcaaTCAGAGATTCTTCCCGGCTATCCCGGGTGAttcgacatgaggcccggatc	
61	67	gggagctcagaataaacgctcaaGTAGTGAAGCTCGTACAGAGGTATTGCGTAttcgacatgaggcccggatc	
69	124	gggagctcagaataaacgctcaaCACATCTGCAGACTGTACCCCACATGGCAAttcgacatgaggcccggatc	
70	128	gggagctcagaataaacgctcaaGAGGGCCGGGTTAGCCTTTTAAGGTTGTGTttcgacatgaggcccggatc	
	Pyrimidine-rich motif		%Y
44	30	gggagctcagaataaacgctcaaACCTCGTACTGCCATCTCTCCCCTCATGTCTtcgacatgaggcccggatc	77
51	126	gggagctcagaataaacgctcaaCGGTTCATCTTTTCTTGTTATTTTTCCACTAttcgacatgaggcccggatc	77
45	35	gggagctcagaataaacgctcaaACACTCACGACTTTTCATCTTTCTCCTTCTtcgacatgaggcccggatc	80
43	2	gggagctcagaataaacgctcaaTGCACCTCACCTCCTTACACTTTCCTTCTTTtcgacatgaggcccggatc	83
49	87	gggagctcagaataaacgctcaaACCCTACTCTCCACTCACATCTTCTTCCCCttcgacatgaggcccggatc	83
50	103	gggagctcagaataaacgctcaaTACCTCACACTCTCTTAATCTCTTCTCTTCttcgacatgaggcccggatc	83
46	36	gggagctcagaataaacgctcaaAACCCTTCTTCACTCTTCTCGCTCTCCTTTTtcgacatgaggcccggatc	87
48	69	gggagctcagaataaacgctcaaGCACTTCTCACTATTCCTTCCTTCTCTCTttcgacatgaggcccggatc	87
47	59	gggagctcagaataaacgctcaaCCCTTCCAATTCCTCTTACTCCTCTCTCCTttcgacatgaggcccggatc	90

TABLE 2

Glioblastoma Ligand Sequences

Sequences: (fixed regions not shown)

Ligand NO:	Random Region	
GBI.1	GGCTGCTGAGTCCAGGGGCGATAACGGGCTTTG	74
GBI.2	GGCTGCTGAGTCCAGGGGCGATAACGGGCTTTG	75
GBI.120	GGCTGCTGAGTCCAGGGGCGATAACGAGCTTTC	76
GBI.140	GGCTGCTGAGGCCAGGGGCGATAACCGCACTTT	77
GBI.162	GGCTGCTGAGTCCAGGGGCGATAACGGCCTTTC	78
GBI.4	TAGC GAACACAGGGGNCCACAACTGGCTATCTCT	79
GBI.8	TAGCAGAACACAGGGGNCCACAACTGGCTATCTC	80
GBI.33	TAGGCGAACACAGGGGTCCACAACTGGCTATCCC	81
GBI.124	TAGC GAACACAGGG TCAACAGCTCACACGGCC	82
GBI.125	TAGC GAACGARCGGTGCCCTGCTCTCAACTGGTTT	83
GBI.99	TAGGCCGGAGGGACTAATAGCTTACAGCGCACTA	84
GBI.76	TAGGCCGGAGGGACTAATAGCTTACAAGGCACTA	85
GBI.42	TAGGAGCGCGAACAACGGGGGAGGTCTCACACTG	86
GBI.23	TAGGGGGNGNNATACAACAGGTCGGTCACAACTG	87
GBI.75	TAGGGCGGAGNGNGGCGGTCATCCTGGNNACACTC	88
GBI.27	AGGCAGAAGTGAGCTTGGGCTCGCAACTCTCTCC	89
GBI.29	AGGCNGTAG GNGCTAGGGNGNACTCGTATTCCTC	90
GBI.101	AGGCAGCAGTGA CTTGGA CGACAACAGCTATGTC	91
GBI.156	AGGCAGTAGTGA CTTGGGCGCAGAGGAGGGTAGT	92
GBI.189	AGGGCGCAGGG TCTAGGGCANCCAACAGCTATTG	93
GBI.145	AGGCGAAGGGN CTAGGGTGNACAGCAGCGGTGG	94
GBI.10	NNNAGAGGGAAGACTTTAGGTTCGGTTCACGTCC	95
GBI.36	NNNAGAGGGAAGAC TTAGGTTCGGTTCACGTCC	96
GBI.41	CCCAGAGGGAAGACTTTAGGTTCGGTTCACGTCCC	97
GBI.73	NCCAGAGGGNAGACTTTAGGTTCGGTTCACGTCC	98
GBI.132	NNNAGAGGGAAGGCTTTAGGTTCGGTTCACGTCC	99
GBI.170	NNNAGAGGGAAGACTTTAGGTTCGGTTCACGTTC	100
GBI.181	NNNAGAGGGNAGACTTTAGGTTCGGTTCACGTCC	101
GBI.14	GTGTGCAACAGAGCAGNNNTTGTCTAACATCACTT	102
GBI.13	GGGGCGAACAGCAGCTACTCACAACATGTCCGGC	103
GBI.26	GTGGCGAACACGGGTCAAGGGCTTCACAATCTG	104
GBI.35	ATGGCGAACACAGCAACTCGCTCACAACTCTCTCC	105
GBI.38	GTAGGCGAACACAGGTTGAGGCTTACACAGGGNT	106
GBI.43	AGCGAACAACTGACTGACGGCAGGGTCAACACNNC	107
GBI.52	TACGAACAACAGCATTCACACAGGCCTTTTTGTT	108
GBI.183	AGCGAGCAACATCTTTCGCAACAGGTTTGGTTCC	109
GBI.62	TTGGCGAACACAGCAACTCGCTCACAACTATCTT	110
GBI.6	AGGTTGGGTAGGTTGG TGGAGGCGAACGTACCAA	111
GBI.58	AGGTTGGGTAGGTTGG TGGAGGCGAACGTCCTAA	112
GBI.182	AGGTTGGGTAGGCTGG TGGAGGCGNACGTCCCAT	113
GBI.141	AGGTTCGC AGGCTGGCTGGAGGCGCGCGACCCAA	114
GBI.37	GGTTTGACCG TAACAA TTGTTAAA GCTCCGGGNN	115
GBI.61	GGTCTGATCG TAACAA TTGTTAAA GCTCCGGGNC	116
GBI.86	GGTTTGATCTCTAACAA TTGTTAAA GCTCCAGGC	117
GBI.94	GGTCTGATCGCTAACAA TTGTTAAA GCTCCGGGGC	118
GBI.104	GGTCTGATCG TAACAAATTGTTAAAAGCTCCGGGCC	119
GBI.119	GGTTTG TCG TAACAA TTGTTAAA GCTCCGGGAC	120

GBI.171	GGTCTGATCG TAACAG TTGTTAAAAGCTCCGGGCG	121
GBI.187	GGTCTGATCG TAACAA TTGTTAA GCTCCGGGCG	122
GBI.18	CCGCCAAGGGAGCTCTCCGAGCTCGGCGCCACTC	123
GBI.60	NCNNCNAAGGAAGATCTCCGAGTTCGGCGTCACTG	124
GBI.68	CTGCCGGGGAAGATCTCCGAGTTCGGCGTCACTG	125
GBI.69	CCGCCAAGGAAGATCTCCGAGTTCGGCGTCACTG	126
GBI.89	CNGCNAAGGAAGATCTCCGAGTTCGGCGTCACTG	127
GBI.123	CNGCCAAGGAAGATCTCCGAGTTCGGCGTCACTA	128
GBI.185	CNNCNAAGGAAGATCTCC AGTTCGGCGTCACTG	129
GBI.188	CNGCNAAGGAAGATCTCCGAGTTCGGNGTTACTG	130
GBI.16	AGACCGTAGGG TTCGGGAGCGATAAACAGTCGTT	131
GBI.126	AGACCGTAGGGGCTTGGGCCA TCAACTGGCGCGG	132
GBI.114	AGACGGTAGCGCCTTGAGTGAATCAATCAGNAGTAA	133
GBI.129	AGACCGTTGGGACTATA GGCGAACACCAGCTACCA	134
GBI.164	AGACGGTAGCCC TTAACGGCGAACAACGCGTTT	135
GBI.70	AGACTGT AGAGACTTGATGGGTCGCAACCGTCA	136
GBI.79	AGACTGT AGAGGCTA GGGTAACAACGGCTCGTTT	137
GBI.90	AGACTGTGAGAGACTA GGCGAGAAACGGGGTTCTC	138
GBI.130	AGACTGT AGAGGCTA GGGCATCAACAGTTCTTCC	139
GBI.154	AGACTG GAGAGACTA GGCGAGAACCGGGGCGC	140
GBI.22	AGAGAGGAGAACTTAT AGGAAACAACGGTCGGC	141
GBI.157	AGACTGTAGAGGCTA GGGTAACAACGGCTCGTCTG	142
GBI.158	AGACTGTTGAGACTAACTGCGAACAACTGC TGTA	143
GBI.190	AGAGCTGTTGACACTAACGCGAACAACAAC TGTA	144
GBI.66	TGGAGGCGATACTTGGCGAACAACAGGGGCTGTA	145
GBI.74	ATGCCGAACAACAGTCTGAACAACAGGTC TGTAT	146
GBI.107	TAGAGCGAATACTTGGCGGAACAACAGGGC TGTA	147
GBI.178	GGACTGTAGAGACCAGTGGAACAACAGATCG GTA	148
GBI.118	TGGAGGCGAA TCTGGCGAGACAACAGCTTTATCTC	149
GBI.137	TGGAGGCGAAGTCTGGCGA ACAAGCGCTTTATCTC	150
GBI.142	TGGAGGCGAA TCTGTCGA ACAACACGTTTATCCC	151
GBI.32	GT CGGAGNAAACTATGTGTTTTAGAGCCATCCC .	152
GBI.167	GTACGGAGAAAACTATGTGTTTTAGAGCCATCCC	153
GBI.184	GTACGGCGCAAACAATGTGTTTTAGAGCNACTCC	154
GBI.34	GTGTAGACTGCAGAGACTGCCAGTGATCTCTCCC	155
GBI.45	GTGTAGACTGCAGAGACTGCCAGTGCTCTCTCCC	156
GBI.72	TTGGGGCGAACACAGGTTGAGGCTTACACAGGGTT	157
GBI.102	AGTAGGCGNACACAGGTTGAGGCTTACACAGGGTT	158
GBI.49	GAACAGGCNNN TTACCTCTGTGGCCGTTTATCCCTC	159
GBI.67	CAGCCCNCCTTACCTCTGT GCAGTTTATCCCTCT	160
GBI.9	AGACATGGACACTAGGGGACACTGCAGCCAACTT	161
GBI.31	AGACA GGAGTGACTTGGCAGCTNACAGACGCTTC	162
GBI.95	GAGACA GGACTGACTTGGCAGCTCACAG CGCTTC	163
GBI.11	TAGTGGCGAACGACAGACTCTCACACACACAGGCTTG	164
GBI.19	TAAGTGGCGAACGACAG CTCTCACACACA GGCTTG	165
GBI.3	TAGTTCCTTGCTTATTCTTGCTTCCCTTGTCTG	166
GBI.5	AGCACTGAGATACGCTTATTCTTGTCTCCGGGCTTGT	167
GBI.15	GAGGACGATCAACAGCGACTTATTCTCACAACTG	168

```
GBI.17                    TCCCGCTTATTCTTGTCTCAGCTTATTATTCTTGT  169
GBI.40                GTGGNNNAAATTCNCTTATTCTTGTCTCTCGTGGT  170
GBI.50                    ACCAGTACGATTATTCTTGTCTCCCTGNNTTNNNT  171
GBI.59                   GGTGGTTGAGCTTATTCTTGTCTCGATTTGCACGTGT  172
GBI.78                    ACCTTGCGGCTTATTCTTGTCTCGCTTCTTCTTGT  173
GBI.80                  AGTTGTTGTCCGCGTTTCTTGTCTCCCTTTTCCT  174
GBI.81                   TAGTCCCTTGCTTATTCTTGTCTTCCCTTGTCTG  175
GBI.82                    ACCTTCCGGCTTATTCTTGTTCTCTGCTTATTCTTGT  176
GBI.85                        GTCGCTTATTCTTGTCTCCCTCTTATTCTTGTCCC  177
GBI.103                 AGCACGAGATACGCTTATTCTTGTCTCCGCGCTTCT  178
GBI.108                   TGTGTTGTTGTTCTTTGTGTCATCCCTGTTCCTC  179
GBI.111               TAGTGCCTGGGACGCTTATTCTTGTCTCCGGGGNCTA  180
GBI.39                  GGAGGCGCTTGTGTCTTGTTCCCTTGTGTGTCTC  181
GBI.163                 GTGGGGTTGTTGTCTTATTCTTGTCTCCGG  182
GBI.166                   AGTCCCCGCTTATTCTTGTCTCCCTTATCGCG  183
GBI.169                     ACACGCTTATTCTTGTCTCCACTTATTCTTGT  184
GBI.174                   GTTGTCGCTTATTCTTGTCTCTGTCTGTTTTGTC  185
GBI.177                 AGAGTGGGGGGCGCTTATTCTTGTCTCCACTCGCTTGT  186
GBI.179                 GACACCCGCCGCGCTTATTGTTGTCTCCNNNCTTTC  187
GBI.191                   GTTGTCGCTTATTCTTGTCTCCCATCCTCTACTC  188
GBI.180                  AGCCGTGTCCAGCTTATTCTTGTCTCCTNNCTTC  189

GBI.24      GGTTGTGTGACTTCTATTTGNNTTTCGTGTCCC  190
GBI.51      GTCGCTGTGTACCGTTTTTTTCTTGTTTGCCTGTC  191
GBI.71      GGTAGGTCCTTTTCTGTCTTCCTTGTTCTCTCGC  192
GBI.77      TGTCTGTCCGTTCTTTTTGTCTGTGTTTTCCCN  193
GBI.83      GTACCTGTTGTCAGCTTTTACCCTTCGTTCCTC  194
GBI.87      AGTCGCGATTCTATTTTTCACTTTCTGTTGTTGC  195
GBI.88      GTTGCCGTATCCTTGTGGAGTTTTCGTTTCTCCC  196
GBI.91      GTTGGTCNGTTCCTTTCTCTGTTGTTCTCCTC  197
GBI.109     TAGTCCCGCGGCTTATTTTTGTCTCCGTTCCGTT  198
GBI.115     AGTCCCTCNNNNATCCTTTTGTTGTCTTGCTGTC  199
GBI.116     TGTGTGTGTGTCGGTGGTTTTTTGTCTTCCTTTTGC  200
GBI.117     GTGTCCGTTGTTCGCGTTTTGTGNCCTGTTTTTCC  201
GBI.133     AGAAGCCTTGTCGTCTTTCCGTTTCTTCTTGTC  202
GBI.186     ACCGGTAGGAGTCCGTTTTTGTTTGCACTATGCC  203
GBI.175     ACCCNACTGTGATGTTCGTGTTTTGTTCCTCCNC  204

GBI.20      GGTCACACCAGTCACAGCACCTACGTCCTGCCCTC  205
GBI.21      GTAGTGGAACCGACTAGCGGGGTGAAGACTCCTC  206
GBI.25      TAGCCCACAGCAATTTTAGTCTGAGTTCCGTC  207
GBI.30      AGGCTGCCGTAAGCTTTGGGAATTGGCCTGCTGC  208
GBI.53      TGGAGGCGAATCTGGCGAACAACAGCCTTATCTC  209
GBI.54      GAGGCTGTAGAGGCTGACTGCGCGCAGCTGCTGTG  210
GBI.57      GAGGCGAGACAGGGTAGCACCTCACAACATGC  211
GBI.65      TGGACTGGAGAGACCTTAGGAGTCATAACTCTCTC  212
GBI.98      GACTGAAGAGCTCAGAGGCGATACAGGCCGCTGT  213
GBI.106     AAGACAGCAGTGGCTAGGGCGATAACTGTCACCAC  214
GBI.110     GACCGCAGGGTTCGGGAGCGATAAACTAGACCTT  215
GBI.112     CATGCGGGTTTGTCCGGACCTCAGCAACAGCTAC  216
GBI.113     GAAGGCGNANACAGGAGGAAAGGCTNACACCTATC  217
GBI.121     GACTGTAGAGACAGGACGTACAATAGGCTCACTC  218
GBI.122     GTTGCATTCCAGGACCGTTCTGTCNGTACCTCGCGC  219
GBI.127     ATGGGGGCGAACCTTTGCGCTCACAACCTACCTGC  220
GBI.128     GAACGACGGGACAGGGCTGAAAACAGGCAGCTAC  221
GBI.131     TGCGCGGTGTTGCNCTTTGTTCTATTCTCCTGTC  222
GBI.135     TGAACCACAAGCCCCAACTAACAACACCCTGC  223
GBI.143     AGGGTGAGATCCAGGGCGCGCTACGTGCGTGTC  224
GBI.147     ACCGCGACTCTTTGCGTACTTCTTGGTCTTCCGCCT  225
GBI.151     TGGGCGAAGGGTCTTGGACGAGGACAGGCGC  226
GBI.165     AGGTCACCGTTATCTCTTCCTGTTGCTCTTTCGC  227
```

GBI.168	AGTCAAACCCCTCTACGCTGTTGTTGATGTCTCCC	228
GBI.172	TAGGCAGAACTCACTAAAAGGTCCAACTGGTTCC	229
GBI.173	TGGACAGGACTCACCTACAAGGCTTACAACGCAT	230
GBI.176	GTAGACTGTAGAGTTACGGCGCGACTACAACGCT	231
GBI.192	AGGCGGTAGCTACTAACATATCACAACATCTTAC	232

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: JENSEN, KIRK
CHEN, HANG
MORRIS, KEVIN
STEPHENS, ANDREW
GOLD, LARRY

(ii) TITLE OF INVENTION: SYSTEMATIC EVOLUTION OF LIGANDS BY EXPONENTIAL ENRICYMENT: TISSUE SELEX

(iii) NUMBER OF SEQUENCES: 240

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Swanson & Bratschun, L.L.C.
(B) STREET: 8400 E. Prentice Avenue, Suite 200
(C) CITY: Englewood
(D) STATE: Colorado
(E) COUNTRY: USA
(F) ZIP: 80111

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3 1/2 diskette, 1.44 MB
(B) COMPUTER: IBM pc compatible
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WordPerfect 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US96/_____
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/714;131
(B) FILING DATE: 10-JUNE-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/536,428
(B) FILING DATE: 11-JUNE-1990

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/964,624
(B) FILING DATE: 21-OCTOBER-1992

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/434,425
(B) FILING DATE: 05-MAY-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/437,667
(B) FILING DATE: 05-MAY-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/434,001
(B) FILING DATE: 05-MAY-1995

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/433,585
(B) FILING DATE: 05-MAY-1995
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Barry J. Swanson
(B) REGISTRATION NUMBER: 33,215
(C) REFERENCE/DOCKET NUMBER: NEX30/PCT
(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 793-3333
(B) TELEFAX: (303) 793-3433

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGAGCTCAG AATAAACGCT CAANNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGGAGCTCAG AATAAACGCT CAA 23

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: NOTE: N at positions 1 represents three biotins
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

NGATCCGGGC CTCATGTCGA A 21

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
AACTCAGTGG TAGGTAACGG TT 22

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
GGGAGCTCAG AATAAACGCT CAACTCAGTG GTAGGTAACG GTTCAAGACG 50
GGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
GGGAGCTCAG AATAAACGCT CAACTCAGTG GTAGGTAACG GTTATATCCG 50
GAATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
GGGAGCTCAG AATAAACGCT CAAAACTCAG TATAAGGTAA CGGTTCCAAC 50
CCAGATTCGA CATGAGGCCC GGATC 75

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GGGAGCTCAG AATAAACGCT CAAACTCAGT AATGCCAAGG TAACGGTTCC 50
CTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGAGCTCAG AATAAACGCT CAAACTCAGT AATGCTAGGT AACGGTTCCC 50
TTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGAGCTCAG AATAAACGCT CAAACTCAGT AATGCACCAG TAACGGTTAC 50
ATCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGGAGCTCAG AATAAACGCT CAACTCAGTA GCAAGGTAAC GGTTCAGATC 50
CACTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGAGCTCAG AATAAACGCT CAAGTCATAA CGGTTAGCCA GAGGACCGTG 50
CCTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGGAGCTCAG AATAAACGCT CAACAGGTCG ATCGAGTCAG GTAGGCGCCG 50
AGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY:linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGGAGCTCAG AATAAACGCT CAAGAGGTCG ATCGAGTCAG GTAGGCGCCG 50
AGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGAGCTCAG AATAAACGCT CAACAGGTCG ATTGAGTCAG GTAGGCGCCG 50
AGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGGAGCTCAG AATAAACGCT CAAGGCGTGT CGATGTGGAA TCACAACCTG 50
TCTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGAGCTCAG AATAAACGCT CAAGGTTGTC GACGCATTAT AGCGACATCG 50
TCTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
GGGAGCTCAG AATAAACGCT CAAGTGGAGT CGACACGCTG TGACCTTTGG 50
CATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
GGGAGCTCAG AATAAACGCT CAAGTGAGTC GACACGCCGC GACCTTTGGT 50
ATTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
GGGAGCTCAG AATAAACGCT CAAGTGCGTC GAGGCATTGC AACCTTTGGT 50
CTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
GGGAGCTCAG AATAAACGCT CAATAGACCG TCGATGCTTG CAACTTTACG 50
TATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
GGGAGCTCAG AATAAACGCT CAATAGTTGC CCACCGTTGT CCAATTGATC 50
GTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGGAGCTCAG AATAAACGCT CAATGGTTGC CCATCGTTGT CCAATTGATC 50
GTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGGAGCTCAG AATAAACGCT CAATGTTGCC CATTCGTCGT CCAAGTGAAC 50
GTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGAGCTCAG AATAAACGCT CAATGAATTG CCCAACGTCG CCCGAATGAT 50
GCGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 74 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGGAGCTCAG AATAAACGCT CAAAGGCGGT GTTACTTCTC ACGAATTGAG 50
GAAGTTCGAC ATGAGGCCCG GATC 74

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGGAGCTCAG AATAAACGCT CAAAGCGTTG TTACTTCTCA CGAATTGAGG 50

AAGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
GGGAGCTCAG AATAAACGCT CAATGAGAGG GGCAACCTTG AGTCTTTCAT 50
GCCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
GGGAGCTCAG AATAAACGCT CAAAGCAGCG GGCAACCTTG AGTATTTCAT 50
GCTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
GGGAGCTCAG AATAAACGCT CAAACCCGGG CAACCGTTCG GTCTTTCAGT 50
CTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
GGGAGCTCAG AATAAACGCT CAACATCGTT GACACCCTCG TGTGCTTCAG 50
GTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
GGGAGCTCAG AATAAACGCT CAACATCGCT TGACAGCTGT GCTGCTTCAG 50
TTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
GGGAGCTCAG AATAAACGCT CAAGGGTGAT CGAAGCCTAG GTGAGCTTGA 50
GCCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
GGGAGCTCAG AATAAACGCT CAAGGGTGTC CGAGCATCCG TAGCTTGAGT 50
CGTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
GGGAGCTCAG AATAAACGCT CAAACGAATC GCATTGCCCA ACGTTGCCCA 50
AGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:
GGGAGCTCAG AATAAACGCT CAACCGAATC GCATTGCCCA ACGTTGCCCA 50
AGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:37:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GGGAGCTCAG AATAAACGCT CAATGTCGGA TAAGTCGCCC AACGTTGCCC 50
ATTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGGAGCTCAG AATAAACGCT CAAGTGGAGC GATTCGCGAA AATCGACTTG 50
CATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGGAGCTCAG AATAAACGCT CAACTGGAGC GATTCGGAAA ATCGACTTGC 50
ATTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGGAGCTCAG AATAAACGCT CAACATCTGG ATGTTCAACC TTCTGGTCTT 50
GCGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
GGGAGCTCAG AATAAACGCT CAACTACCCG GTTGAACCTT CGCTCTTGCG 50
TAGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:42:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
GGGAGCTCAG AATAAACGCT CAATGCTCCC CGAAACCCTA TTTCTTGCTG 50
CTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:
GGGAGCTCAG AATAAACGCT CAATGCACCT CACCTCCTTA CACTTTCCTT 50
CTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:44:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
GGGAGCTCAG AATAAACGCT CAAACCTCGT ACTGCCATCT CTCCCCTCAT 50
GTCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
GGGAGCTCAG AATAAACGCT CAAACACTCA CGACTTTTCA TCTTTCTCCT 50
TCTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:46:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GGGAGCTCAG AATAAACGCT CAAAACCCTT CTTCACTCTT CTCGCTCTCC 50
TTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGGAGCTCAG AATAAACGCT CAACCCTTCC AATTCCTCTT ACTCCTCTCT 50
CCTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGGAGCTCAG AATAAACGCT CAAGCACTTC TCACTATTCC TTCCTTCTCT 50
CTCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGGAGCTCAG AATAAACGCT CAAACCCTAC TCTCCACTCA CATCTTCTTC 50
CCCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:50:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GGGAGCTCAG AATAAACGCT CAATACCTCA CACTCTCTTA ATCTCTTCTC 50

TTCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:51:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 74 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:
GGGAGCTCAG AATAAACGCT CAACGGTTCA TCTTTTCTTG TTATTTTTCC 50
ACTATTCGAC ATGAGGCCCG GATC 74

(2) INFORMATION FOR SEQ ID NO:52:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:
GGGAGCTCAG AATAAACGCT CAAGTGGCCT CAAACTGCTA GGAGTAAACA 50
TGTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:53:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:
GGGAGCTCAG AATAAACGCT CAATAGGGGT AGGGCGCAAT ATTCACCGGG 50
CCTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:54:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:
GGGAGCTCAG AATAAACGCT CAAGGAGCGC GATACGTTTA CTTCTGATCA 50
TGTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:55:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:
GGGAGCTCAG AATAAACGCT CAAAGAGGAG TCTTGCTGTC CGTACACAGC 50
TTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:56:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:
GGGAGCTCAG AATAAACGCT CAATCCCTTG AACCATCGGT CTTGCGTTCC 50
ATGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:57:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:
GGGAGCTCAG AATAAACGCT CAAACAAGAG GGTCTTGCCG CACCATTCGG 50
CTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:58:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:
GGGAGCTCAG AATAAACGCT CAAACGAGTT ACAGCCACCC ATGCTGTCGG 50
TGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:59:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:
GGGAGCTCAG AATAAACGCT CAAGACAGCG TGATTCCTCC GCTCTGCTGC 50
TATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:60:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGGAGCTCAG AATAAACGCT CAACGGGACC TTGAGTATTC CTCATTATCG 50
TTCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:61:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GGGAGCTCAG AATAAACGCT CAAGTAGTGA AGCTCGTACA GAGGTATTGC 50
GTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:62:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGGAGCTCAG AATAAACGCT CAAAGCCGAA TTAGTAGCGT ATAGCGTGTT 50
GTGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:63:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GGGAGCTCAG AATAAACGCT CAAGGGCAAT ACACAACACT CTACCTCACC 50
TCATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:64:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:
GGGAGCTCAG AATAAACGCT CAATCAGAGA TTCTTCCCGG CTATCCCGGG 50
TGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:65:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:
GGGAGCTCAG AATAAACGCT CAATAGGCCG GGTGAGCTAC TTCTAGTAGG 50
GTGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:66:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:
GGGAGCTCAG AATAAACGCT CAAGTTGTGA TCCATTAGCG GCACCGCCTC 50
CATTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:67:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:
GGGAGCTCAG AATAAACGCT CAATCCGGAA AGCAACGCAT ACTTCGCATG 50
TCGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:68:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:
GGGAGCTCAG AATAAACGCT CAAGTGAGCG TACCGGAGTG TGTTACCAAT 50
TATTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:69:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GGGAGCTCAG AATAAACGCT CAACACATCT GCAGACTGTA CCCCACATGG 50
CAATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:70:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GGGAGCTCAG AATAAACGCT CAAGAGGGCC GGGTTAGCCT TTTAAGGTTG 50
TGTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:71:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GCCTGTTGTG AGCCTCCTNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNGCCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:72:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(D) OTHER INFORMATION: N at position 1 is fluroscein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

NGCCTGTTGT GAGCCTCCT 19

(2) INFORMATION FOR SEQ ID NO:73:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GGGAGACAAG AATAAGCG 18

(2) INFORMATION FOR SEQ ID NO:74:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:
GCCTGTTGTG AGCCTCCTGG CTGCTGAGTC CAGGGGCGAT AACGGGCTTT 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:75:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:
GCCTGTTGTG AGCCTCCTGG CTGCTGAGTC CAGGGGCGAT AACGGGCTTT 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:76:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:
GCCTGTTGTG AGCCTCCTGG CTGCTGAGTC CAGGGGCGAT AACGAGCTTT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:77:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:
GCCTGTTGTG AGCCTCCTGG CTGCTGAGGC CAGGGGCGAT AACCGCACTT 50
TCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:78:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GCCTGTTGTG AGCCTCCTGG CTGCTGAGTC CAGGGGCGAT AACGGCCTTT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:79:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GCCTGTTGTG AGCCTCCTTA GCGAACACAG GGGNCCACAA CTGGCTATCT 50
CTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:80:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GCCTGTTGTG AGCCTCCTTA GCAGAACACA GGGGNCCACA ACTGGCTATC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:81:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GCCTGTTGTG AGCCTCCTTA GGCGAACACA GGGGTCCACA ACTGGCTATC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:82:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GCCTGTTGTG AGCCTCCTTA GCGAACACAG GGTCAACAGC TCACACGGCC 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:83:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GCCTGTTGTG AGCCTCCTTA GCGAACGARC GGTGCCCTGC TCTCAACTGG 50
TTTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:84:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GCCTGTTGTG AGCCTCCTTA GGCCGGAGGG ACTAATAGCT TACAGCGCAC 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:85:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GCCTGTTGTG AGCCTCCTTA GGCCGGAGGG ACTAATAGCT TACAAGGCAC 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:86:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GCCTGTTGTG AGCCTCCTTA GGAGCGCGAA CAACGGGGGA GGTCTCACAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:87:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GCCTGTTGTG AGCCTCCTTA GGGGGNGNNA TACAACAGGT CGGTCACAAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:88:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GCCTGTTGTG AGCCTCCTTA GGGCGGAGNG NGGCGGTCAT CCTGGNNACA 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:89:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GCCTGTTGTG AGCCTCCTAG GCAGAAGTGA GCTTGGGCTC GCAACTCTCT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:90:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GCCTGTTGTG AGCCTCCTAG GCNGTAGGNG CTAGGGNGNA CTCGTATTCC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:91:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

GCCTGTTGTG AGCCTCCTAG GCAGCAGTGA CTTGGACGAC AACAGCTATG 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:92:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GCCTGTTGTG AGCCTCCTAG GCAGTAGTGA CTTGGGCGCA GAGGAGGGTA 50
GTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:93:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GCCTGTTGTG AGCCTCCTAG GGCGCAGGGT CTAGGGCANC CAACAGCTAT 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:94:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

GCCTGTTGTG AGCCTCCTAG GCGAAGGGNC TAGGGTGNAC AGCAGCGGTG 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:95:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GCCTGTTGTG AGCCTCCTNN NAGAGGGAAG ACTTTAGGTT CGGTTCACGT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:96:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GCCTGTTGTG AGCCTCCTNN NAGAGGGAAG ACTTAGGTTC GGTTCACGTC 50

CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:97:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:
GCCTGTTGTG AGCCTCCTCC CAGAGGGAAG ACTTTAGGTT CGGTTCACGT 50
CCCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:98:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:
GCCTGTTGTG AGCCTCCTNC CAGAGGGNAG ACTTTAGGTT CGGTTCACGT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:99:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:
GCCTGTTGTG AGCCTCCTNN NAGAGGGAAG GCTTTAGGTT CGGTTCACGT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:100:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:
GCCTGTTGTG AGCCTCCTNN NAGAGGGAAG ACTTTAGGTT CGGTTCACGT 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:101:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GCCTGTTGTG AGCCTCCTNN NAGAGGGNAG ACTTTAGGTT CGGTTCACGT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:102:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GCCTGTTGTG AGCCTCCTGT GTGCAACAGA GCAGNNNTTG TCTAACATCA 50
CTTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:103:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

GCCTGTTGTG AGCCTCCTGG GGCGAACAGC AGCTACTCAC AACATGTCCG 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:104:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

GCCTGTTGTG AGCCTCCTGT GGCGAACACG GGTCAAGGGC TTCACAATCT 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:105:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

GCCTGTTGTG AGCCTCCTAT GGCGAACACA GCAACTCGCT CACAACTCTC 50
TCCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:106:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

GCCTGTTGTG AGCCTCCTGT AGGCGAACAC AGGTTGAGGC TTACACAGGG 50
NTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:107:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

GCCTGTTGTG AGCCTCCTAG CGAACAACTG ACTGACGGCA GGGTCAACAC 50
NNCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:108:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

GCCTGTTGTG AGCCTCCTTA CGAACAACAG CATTCACACA GGCCTTTTTG 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:109:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

GCCTGTTGTG AGCCTCCTAG CGAGCAACAT CTTTCGCAAC AGGTTTGGTT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:110:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

GCCTGTTGTG AGCCTCCTTT GGCGAACACA GCAACTCGCT CACAACTATC 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:111:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

GCCTGTTGTG AGCCTCCTAG GTTGGGTAGG TTGGTGGAGG CGAACGTACC 50
AACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:112:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

GCCTGTTGTG AGCCTCCTAG GTTGGGTAGG TTGGTGGAGG CGAACGTCCT 50
AACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:113:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

GCCTGTTGTG AGCCTCCTAG GTTGGGTAGG CTGGTGGAGG CGNACGTCCC 50
ATCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:114:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

GCCTGTTGTG AGCCTCCTAG GTTCGCAGGC TGGCTGGAGG CGCGCGACCC 50
AACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:115:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

GCCTGTTGTG AGCCTCCTGG TTTGACCGTA ACAATTGTTA AAGCTCCGGG 50
NNCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:116:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

GCCTGTTGTG AGCCTCCTGG TCTGATCGTA ACAATTGTTA AAGCTCCGGG 50
NCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:117:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

GCCTGTTGTG AGCCTCCTGG TTTGATCTCT AACAATTGTT AAAGCTCCAG 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:118:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

GCCTGTTGTG AGCCTCCTGG TCTGATCGCT AACAATTGTT AAAGCTCCGG 50
GGCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:119:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

GCCTGTTGTG AGCCTCCTGG TCTGATCGTA ACAAATTGTT AAAAGCTCCG 50

GGCCCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:120:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:
GCCTGTTGTG AGCCTCCTGG TTTGTCGTAA CAATTGTTAA AGCTCCGGGA 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:121:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:
GCCTGTTGTG AGCCTCCTGG TCTGATCGTA ACAGTTGTTA AAAGCTCCGG 50
GCGCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:122:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:
GCCTGTTGTG AGCCTCCTGG TCTGATCGTA ACAATTGTTA AGCTCCGGGC 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:123:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:
GCCTGTTGTG AGCCTCCTCC GCCAAGGGAG CTCTCCGAGC TCGGCGCCAC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:124:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

GCCTGTTGTG AGCCTCCTNC NNCNAAGGAA GATCTCCGAG TTCGGCGTCA 50
CTGCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:125:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

GCCTGTTGTG AGCCTCCTCT GCCGGGGAAG ATCTCCGAGT TCGGCGTCAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:126:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

GCCTGTTGTG AGCCTCCTCC GCCAAGGAAG ATCTCCGAGT TCGGCGTCAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:127:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

GCCTGTTGTG AGCCTCCTCN GCNAAGGAAG ATCTCCGAGT TCGGCGTCAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:128:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

GCCTGTTGTG AGCCTCCTCN GCCAAGGAAG ATCTCCGAGT TCGGCGTCAC 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:129:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

GCCTGTTGTG AGCCTCCTCN NCNAAGGAAG ATCTCCAGTT CGGCGTCACT 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:130:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

GCCTGTTGTG AGCCTCCTCN GCNAAGGAAG ATCTCCGAGT TCGGNGTTAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:131:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

GCCTGTTGTG AGCCTCCTAG ACCGTAGGGT TCGGGAGCGA TAAACAGTCG 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:132:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

GCCTGTTGTG AGCCTCCTAG ACCGTAGGGG CTTGGGCCAT CAACTGGCGC 50
GGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:133:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

GCCTGTTGTG AGCCTCCTAG ACGGTAGCGC CTTGAGTGAA TCAATCAGNA 50
GTAACGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:134:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

GCCTGTTGTG AGCCTCCTAG ACCGTTGGGA CTATAGGCGA ACACCAGCTA 50
CCACGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:135:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

GCCTGTTGTG AGCCTCCTAG ACGGTAGCCC TTAACGGCGA ACAACGCGTT 50
TCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:136:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

GCCTGTTGTG AGCCTCCTAG ACTGTAGAGA CTTGATGGGT CGCAACCGTC 50
ACGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:137:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

GCCTGTTGTG AGCCTCCTAG ACTGTAGAGG CTAGGGTAAC AACGGCTCGT 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:138:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

GCCTGTTGTG AGCCTCCTAG ACTGTGAGAG ACTAGGCGAG AAACGGGGTT 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:139:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

GCCTGTTGTG AGCCTCCTAG ACTGTAGAGG CTAGGGCATC AACAGTTCTT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:140:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

GCCTGTTGTG AGCCTCCTAG ACTGGAGAGA CTAGGCGAGA ACCGGGGCGC 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:141:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

GCCTGTTGTG AGCCTCCTAG AGAGGAGAAC TTATAGGAAA CAACGGTCGG 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:142:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

GCCTGTTGTG AGCCTCCTAG ACTGTAGAGG CTAGGGTAAC AACGGCTCGT 50

CTGCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:143:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:
GCCTGTTGTG AGCCTCCTAG ACTGTTGAGA CTAACTGCGA ACAACTGCTG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:144:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:
GCCTGTTGTG AGCCTCCTAG AGCTGTTGAC ACTAACGCGA ACAACAACTG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:145:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:
GCCTGTTGTG AGCCTCCTTG GAGGCGATAC TTGGCGAACA ACAGGGGCTG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:146:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:
GCCTGTTGTG AGCCTCCTAT GCCGAACAAC AGTCTGAACA ACAGGTCTGT 50
ATCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:147:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

GCCTGTTGTG AGCCTCCTTA GAGCGAATAC TTGGCGGAAC AACAGGGCTG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:148:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

GCCTGTTGTG AGCCTCCTGG ACTGTAGAGA CCAGTGGAAC AACAGATCGG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:149:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

GCCTGTTGTG AGCCTCCTTG GAGGCGAATC TGGCGAGACA ACAGCTTTAT 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:150:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

GCCTGTTGTG AGCCTCCTTG GAGGCGAAGT CTGGCGAACA AGCGCTTTAT 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:151:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

GCCTGTTGTG AGCCTCCTTG GAGGCGAATC TGTCGAACAA CACGTTTATC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:152:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

GCCTGTTGTG AGCCTCCTGT CGGAGNAAAC TATGTGTTTT AGAGCCATCC 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:153:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

GCCTGTTGTG AGCCTCCTGT ACGGAGAAAA CTATGTGTTT TAGAGCCATC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:154:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

GCCTGTTGTG AGCCTCCTGT ACGGCGCAAA CAATGTGTTT TAGAGCNACT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:155:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

GCCTGTTGTG AGCCTCCTGT GTAGACTGCA GAGACTGCCA GTGATCTCTC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:156:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

GCCTGTTGTG AGCCTCCTGT GTAGACTGCA GAGACTGCCA GTGCTCTCTC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:157:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

GCCTGTTGTG AGCCTCCTTT GGGGCGAACA CAGGTTGAGG CTTACACAGG 50
GTTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:158:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

GCCTGTTGTG AGCCTCCTAG TAGGCGNACA CAGGTTGAGG CTTACACAGG 50
GTTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:159:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

GCCTGTTGTG AGCCTCCTGA ACAGGCNNNT TACCTCTGTG GCCGTTTATC 50
CCTCCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:160:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

GCCTGTTGTG AGCCTCCTCA GCCCNCCTTA CCTCTGTGCA GTTTATCCCT 50
CTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:161:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

GCCTGTTGTG AGCCTCCTAG ACATGGACAC TAGGGGACAC TGCAGCCAAC 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:162:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

GCCTGTTGTG AGCCTCCTAG ACAGGAGTGA CTTGGCAGCT NACAGACGCT 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:163:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

GCCTGTTGTG AGCCTCCTGA GACAGGACTG ACTTGGCAGC TCACAGCGCT 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:164:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

GCCTGTTGTG AGCCTCCTTA GTGGCGAACG ACAGACTCTC ACACACACAG 50
GCTTGCGCTT ATTCTTGTCT CCC 73

(2) INFORMATION FOR SEQ ID NO:165:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

GCCTGTTGTG AGCCTCCTTA AGTGGCGAAC GACAGCTCTC ACACACAGGC 50

TTGCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:166:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:
GCCTGTTGTG AGCCTCCTTA GTTCCTTGCT TATTCTTGCT TCCCTTGTCT 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:167:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:
GCCTGTTGTG AGCCTCCTAG CACTGAGATA CGCTTATTCT TGTCTCCGGG 50
CTTGTCGCTT ATTCTTGTCT CCC 73

(2) INFORMATION FOR SEQ ID NO:168:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:
GCCTGTTGTG AGCCTCCTGA GGACGATCAA CAGCGACTTA TTCTCACAAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:169:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:
GCCTGTTGTG AGCCTCCTTC CCGCTTATTC TTGTCTCAGC TTATTATTCT 50
TGTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:170:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:

GCCTGTTGTG AGCCTCCTGT GGNNNAAATT CNCTTATTCT TGTCTCTCGT 50
GGTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:171:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:

GCCTGTTGTG AGCCTCCTAC CAGTACGATT ATTCTTGTCT CCCTGNNTTN 50
NNTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:172:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:

GCCTGTTGTG AGCCTCCTGG TGGTTGAGCT TATTCTTGTC TCGATTTGCA 50
CGTGTCGCTT ATTCTTGTCT CCC 73

(2) INFORMATION FOR SEQ ID NO:173:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:

GCCTGTTGTG AGCCTCCTAC CTTGCGGCTT ATTCTTGTCT CGCTTCTTCT 50
TGTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:174:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:

GCCTGTTGTG AGCCTCCTAG TTGTTGTCCG CGTTTCTTGT CTCCCTTTTC 50
CTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:175:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:

GCCTGTTGTG AGCCTCCTTA GTCCCTTGCT TATTCTTGTC TTCCCTTGTC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:176:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:

GCCTGTTGTG AGCCTCCTAC CTTCCGGCTT ATTCTTGTTC TCTGCTTATT 50
CTTGTCGCTT ATTCTTGTCT CCC 73

(2) INFORMATION FOR SEQ ID NO:177:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:177:

GCCTGTTGTG AGCCTCCTGT CGCTTATTCT TGTCTCCCTC TTATTCTTGT 50
CCCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:178:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:178:

GCCTGTTGTG AGCCTCCTAG CACGAGATAC GCTTATTCTT GTCTCCGCGC 50
TTCTCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:179:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:179:

GCCTGTTGTG AGCCTCCTTG TGTTGTTGTT CTTTGTGTCA TCCCTGTTCC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:180:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:180:

GCCTGTTGTG AGCCTCCTTA GTGCCTGGGA CGCTTATTCT TGTCTCCGGG 50
GNCTACGCTT ATTCTTGTCT CCC 73

(2) INFORMATION FOR SEQ ID NO:181:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:181:

GCCTGTTGTG AGCCTCCTGG AGGCGCTTGT GTCTTGTTCC CTTGTGTGTC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:182:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:182:

GCCTGTTGTG AGCCTCCTGT GGGGTTGTTG TCTTATTCTT GTCTCCGGCG 50
CTTATTCTTG TCTCCC 66

(2) INFORMATION FOR SEQ ID NO:183:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:183:

GCCTGTTGTG AGCCTCCTAG TCCCCGCTTA TTCTTGTCTC CCTTATCGCG 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:184:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:184:

GCCTGTTGTG AGCCTCCTAC ACGCTTATTC TTGTCTCCAC TTATTCTTGT 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:185:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:185:

GCCTGTTGTG AGCCTCCTGT TGTCGCTTAT TCTTGTCTCT GTCTGTTTTG 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:186:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 74 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:186:

GCCTGTTGTG AGCCTCCTAG AGTGGGGGGC GCTTATTCTT GTCTCCACTC 50
GCTTGTCGCT TATTCTTGTC TCCC 74

(2) INFORMATION FOR SEQ ID NO:187:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:187:

GCCTGTTGTG AGCCTCCTGA CACCCGCCGC GCTTATTGTT GTCTCCNNNC 50
TTTCCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:188:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:188:

GCCTGTTGTG AGCCTCCTGT TGTCGCTTAT TCTTGTCTCC CATCCTCTAC 50

TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:189:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:
GCCTGTTGTG AGCCTCCTAG CCGTGTCCAG CTTATTCTTG TCTCCTNNCT 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:190:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:
GCCTGTTGTG AGCCTCCTGG TTGTGTGACT TCTATTTGNN TTTCGTGTCC 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:191:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:
GCCTGTTGTG AGCCTCCTGT CGCTGTGTAC CGTTTTTTTC TTGTTTGCCT 50
GTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:192:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:
GCCTGTTGTG AGCCTCCTGG TAGGTCCTTT TCTGTCTTCC TTGTTCTCTC 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:193:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:

GCCTGTTGTG AGCCTCCTTG TCTGTCCGTT CTTTTTGTCT GTGTTTTCCC 50
NCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:194:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:

GCCTGTTGTG AGCCTCCTGT ACCTGTTGTC AGCTTTTACC CTTCGTTCCT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:195:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:

GCCTGTTGTG AGCCTCCTAG TCGCGATTCT ATTTTTCACT TTCTGTTGTT 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:196:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:

GCCTGTTGTG AGCCTCCTGT TGCCGTATCC TTGTGGAGTT TTCGTTTCTC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:197:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:

GCCTGTTGTG AGCCTCCTGT TGGTCNGTTC CTTTCTCTGT TGTTCTCCTC 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:198:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:

GCCTGTTGTG AGCCTCCTTA GTCCCGCGGC TTATTTTTGT CTCCGTTCCG 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:199:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:

GCCTGTTGTG AGCCTCCTAG TCCCTCNNNN ATCCTTTTGT TGTCTTGCTG 50
TCCGCTTATT CTTGTCTCCC 70

2) INFORMATION FOR SEQ ID NO:200:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:

GCCTGTTGTG AGCCTCCTTG TGTGTGTGTC GGTGGTTTTT TGTCTTCCTT 50
TTGCCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:201:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:

GCCTGTTGTG AGCCTCCTGT GTCCGTTGTT CGCGTTTTGT GNCCTGTTTT 50
TCCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:202:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:

GCCTGTTGTG AGCCTCCTAG AAGCCTTGTC GTCTTTCCGT TTCTTCTTGT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:203:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:

GCCTGTTGTG AGCCTCCTAC CGGTAGGAGT CCGTTTTTGT TTGCACTATG 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:204:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:

GCCTGTTGTG AGCCTCCTAC CCNACTGTGA TGTTCGTGTT TTGTTCCTCC 50
NCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:205:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:

GCCTGTTGTG AGCCTCCTGG TCACACCAGT CACAGCACCT ACGTCCTGCC 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:206:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:

GCCTGTTGTG AGCCTCCTGT AGTGGAACCG ACTAGCGGGG TGAAGACTCC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:207:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:207:

GCCTGTTGTG AGCCTCCTTA GCCCACAGCA ATTTTAGTCT GAGTTCCGTC 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:208:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:

GCCTGTTGTG AGCCTCCTAG GCTGCCGTAA GCTTTGGGAA TTGGCCTGCT 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:209:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:

GCCTGTTGTG AGCCTCCTTG GAGGCGAATC TGGCGAACAA CAGCCTTATC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:210:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:

GCCTGTTGTG AGCCTCCTGA GGCTGTAGAG GCTGACTGCG CGCAGCTGCT 50
GTGCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:211:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:

GCCTGTTGTG AGCCTCCTGA GGCGAGACAG GGTAGCACCT CACAACATGC 50

CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:212:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:
GCCTGTTGTG AGCCTCCTTG GACTGGAGAG ACCTTAGGAG TCATAACTCT 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:213:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:
GCCTGTTGTG AGCCTCCTGA CTGAAGAGCT CAGAGGCGAT ACAGGCCGCT 50
GTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:214:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:
GCCTGTTGTG AGCCTCCTAA GACAGCAGTG GCTAGGGCGA TAACTGTCAC 50
CACCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:215:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215:
GCCTGTTGTG AGCCTCCTGA CCGCAGGGTT CGGGAGCGAT AAACTAGACC 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:216:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216:
GCCTGTTGTG AGCCTCCTCA TGCGGGTTTG TCCGGACCTC AGCAACAGCT 50
ACCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:217:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:217:
GCCTGTTGTG AGCCTCCTGA AGGCGNANAC AGGAGGAAAG GCTNACACCT 50
ATCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:218:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:218:
GCCTGTTGTG AGCCTCCTGA CTGTAGAGAC AGGACGTACA ATAGGCTCAC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:219:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:219:
GCCTGTTGTG AGCCTCCTGT TGCATTCCAG GACCGTTCTG TCNGTACCTC 50
GCGCCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:220:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220:
GCCTGTTGTG AGCCTCCTAT GGGGGCGAAC CTTTGCGCTC ACAACCTACC 50
TGCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:221:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221:

GCCTGTTGTG AGCCTCCTGA ACGACGGGAC AGGGCTGAAA ACAGGCAGCT 50
ACCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:222:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:222:

GCCTGTTGTG AGCCTCCTTG CGCGGTGTTG CNCTTTGTTC TATTCTCCTG 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:223:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:

GCCTGTTGTG AGCCTCCTTG AACCACAAGC CCCAACTAAC AACACCCTGC 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:224:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:224:

GCCTGTTGTG AGCCTCCTAG GGTGAGATCC AGGGCGCGCT ACGTGCGTGT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:225:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:225:

GCCTGTTGTG AGCCTCCTAC CGCGACTCTT TGCGTACTTC TTGGTCTTCC 50
GCCTCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:226:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:226:

GCCTGTTGTG AGCCTCCTTG GGCGAAGGGT CTTGGACGAG GACAGGCGCC 50
GCTTATTCTT GTCTCCC 67

(2) INFORMATION FOR SEQ ID NO:227:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:227:

GCCTGTTGTG AGCCTCCTAG GTCACCGTTA TCTCTTCCTG TTGCTCTTTC 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:228:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:228:

GCCTGTTGTG AGCCTCCTAG TCAAACCCCT CTACGCTGTT GTTGATGTCT 50
CCCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:229:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:229:

GCCTGTTGTG AGCCTCCTTA GGCAGAACTC ACTAAAAGGT CCAACTGGTT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:230:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:230:

GCCTGTTGTG AGCCTCCTTG GACAGGACTC ACCTACAAGG CTTACAACGC 50
ATCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:231:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:231:

GCCTGTTGTG AGCCTCCTGT AGACTGTAGA GTTACGGCGC GACTACAACG 50
CTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:232:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:232:

GCCTGTTGTG AGCCTCCTAG GCGGTAGCTA CTAACATATC ACAACATCTT 50
ACCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:233:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) FEATURE:
(D) OTHER INFORMATION: N at position 1 is fluroscein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:233:

NGCCTGTTGT GAGCCTCCT 19

(2) INFORMATION FOR SEQ ID NO:234:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:234:

GGGAGACAAG AATAAGCG 18

(2) INFORMATION FOR SEQ ID NO:235:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:235:
GCCTGTTGTG AGCCTCCTNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:236:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:236:
AACTCAGTAA TGCCAAGGTA ACGGTT 26

(2) INFORMATION FOR SEQ ID NO:237:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:237:
CGAATCGCAT TGCCCAACGT TGCCCAAGAT TCG 33

(2) INFORMATION FOR SEQ ID NO:238:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:238:
CGCTCAATAG TTGCCCACCG TTGTCCAATT GAGCG 35

(2) INFORMATION FOR SEQ ID NO:239:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:239:

GTCGAGGCAT TGCAACCTTT GGTCTTTCGA C 31

(2) INFORMATION FOR SEQ ID NO:240:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:240:
GGGCAACCTT GAGTATTTCA TGCTTCGACA TGAGGCCCG 39

CLAIMS:

1. A method for identifying nucleic acid ligands and nucleic acid ligand sequences to a tissue target comprising:

a) preparing a candidate mixture of nucleic acid sequences;

b) contacting said candidate mixture of nucleic acids with said tissue, wherein nucleic acids having an increased affinity to the tissue relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

c) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

d) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to said tissue, whereby nucleic acid ligands of said tissue may be identified.

2. The method of Claim 1 further comprising:

e) repeating steps b), c) and d).

3. The method of Claim 1 wherein said tissue is selected from the group consisting of a cell, a subcellular component, an aggregate of cells, a collection of cells, an aggregate of macromolecules.

4. The method of Claim 1 wherein said candidate mixture is comprised of single-stranded nucleic acids.

5. The method of Claim 4 wherein said single-stranded nucleic acids are ribonucleic acids.

6. The method of Claim 4 wherein said single-stranded nucleic acids are deoxyribonucleic acids.

7. The method of Claim 1 wherein said tissue is selected from the group consisting of red blood cells ghosts, glioblastoma, and lymphoma.

8. A nucleic acid ligand to a tissue target identified according to the method of Claim 1.

9. A purified and isolated non-naturally occurring nucleic acid ligand to tissue.

10. The purified nucleic acid ligand of Claim 9 which is a non-naturally occurring nucleic acid ligand having a specific binding affinity for a tissue target molecule, such target molecule being a three dimensional chemical structure other than a polynucleotide that binds to said nucleic acid ligand through a mechanism which predominantly depends on Watson/Crick base pairing or triple helix binding, wherein said nucleic acid ligand is not a nucleic acid having the known physiological function of being bound by the target molecule.

11. The nucleic acid ligand of Claim 9 which is a deoxyribonucleic acid ligand.

12. The nucleic acid ligand of Claim 9 which is a ribonucleic acid ligand.

13. The nucleic acid ligand of Claim 9 wherein said tissue is selected from the group consisting of a cell, a subcellular component, an aggregate of cells, a collection of cells, an aggregate of macromolecules.

14. The nucleic acid ligand of Claim 13 wherein said subcellular component is a red blood cell ghost.

15. The nucleic acid ligand to a red blood cell ghost of Claim 14 wherein said ligand is a DNA ligand selected from the group consisting of the nucleotide sequences set forth in Table 1, or the corresponding RNA sequences thereof or the corresponding complementary sequences thereof.

16. The nucleic acid ligand of Claim 15 wherein said ligand is selected from the group consisting of SEQ ID NOS:4-70.

17. A purified and isolated non-naturally occurring DNA ligand to a red blood cell ghost, wherein said ligand is substantially homologous to and has substantially the same ability to bind said red blood cell ghost as a ligand selected from the group consisting of the sequences set forth in Table 1 or the corresponding RNA sequences thereof or the corresponding complimentary sequences thereof.

18. A purified and isolated non-naturally occurring DNA ligand to a red blood cell ghost, wherein said ligand has substantially the same structure and the same ability to bind said red blood cell ghost as a ligand selected from the group consisting of the sequences set forth in Table 1 or the corresponding RNA sequence thereof or the corresponding complementary sequences thereof.

19. The nucleic acid ligand of Claim 13 wherein said cell in a tumor cell.

20. The nucleic acid ligand of Claim 19 wherein said tumor cell is a glioblastoma.

21. The nucleic acid ligand to a glioblastoma of Claim 20 wherein said ligand is a DNA ligand selected from the group consisting of the nucleotide sequences set forth in Table 2, or the corresponding RNA sequences thereof or the corresponding complementary sequences thereof.

22. The nucleic acid ligand of Claim 21 wherein said ligand is selected from the group consisting of SEQ ID NOS:74-232.

23. A purified and isolated non-naturally occurring DNA ligand to a glioblastoma, wherein said ligand is substantially homologous to and has substantially the same ability to bind said glioblastoma as a ligand selected from the group consisting of the

sequences set forth in Table 2 or the corresponding RNA sequences thereof or the corresponding complimentary sequences thereof.

24. A purified and isolated non-naturally occurring DNA ligand to glioblastoma, wherein said ligand has substantially the same structure and the same ability to bind said glioblastoma as a ligand selected from the group consisting of the sequences set forth in Table 2 or the corresponding RNA sequence thereof or the corresponding complementary sequences thereof.

25. The nucleic acid ligand of Claim 19 wherein said tumor cell is a lymphoma.

26. A method for identifying a macromolecule component of a tissue comprising:

a) identifying a nucleic acid ligand to a new epitope of said macromolecule by the method of Claim 1;

b) purifying said macromolecule component of said tissue away from the remainder of said tissue on the basis of affinity between said new epitope and said nucleic acid ligand; and

c) identifying said macromolecule.

27. The method of Claim 26 wherein said macromolecule is selected from the group consisting of a protein, lipid and carbohydrate.

28. A purified macromolecule identified according to the method of Claim 26.

29. The purified macromolecule of Claim 28 which is selected from the group consisting of a protein, lipid and carbohydrate.

30. The purified macromolecule of Claim 29 which is a tumor associated antigen.

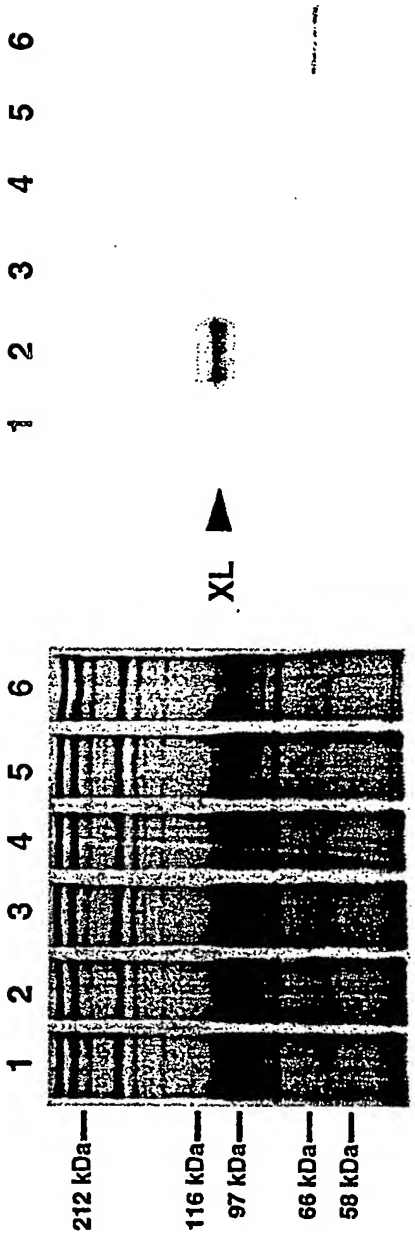


Figure 1

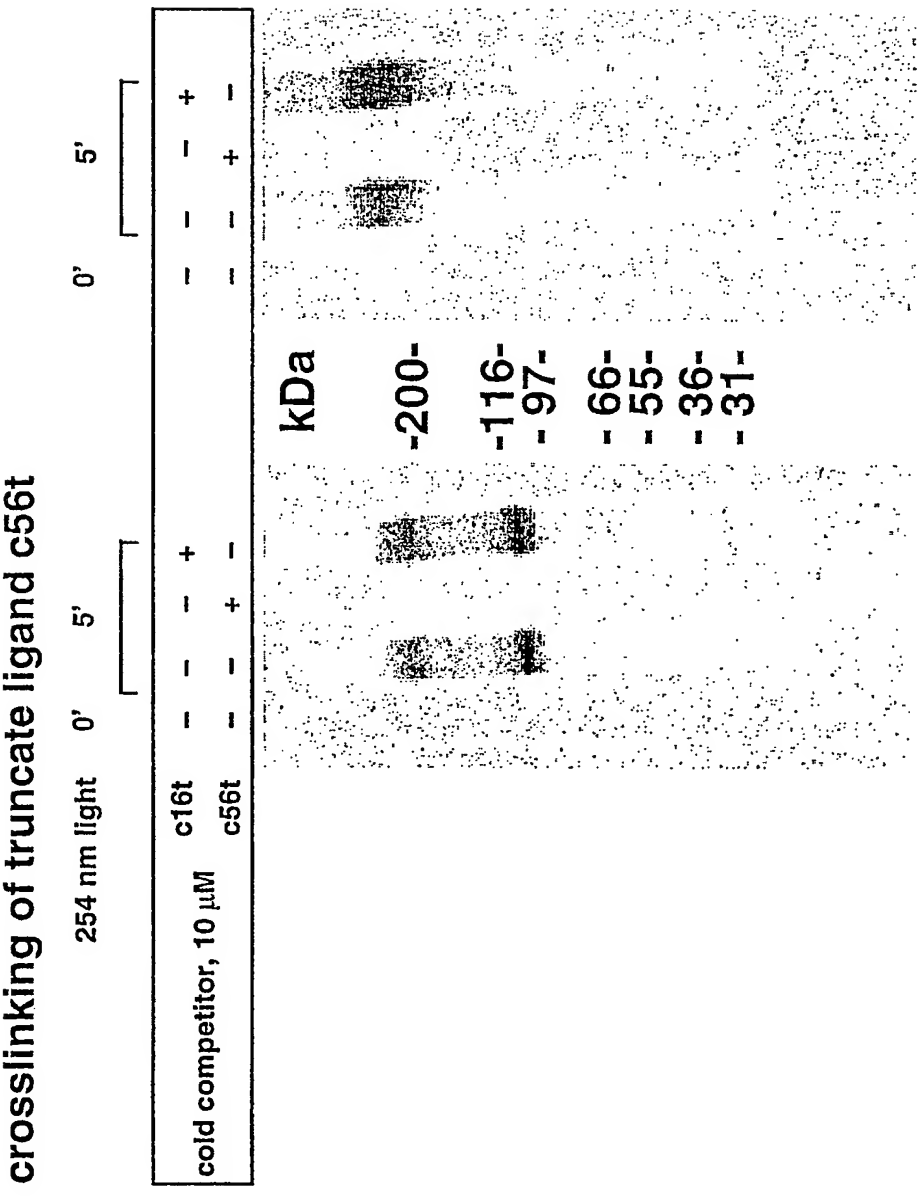

crosslinking of truncate ligand c56t
254 nm light
0'
5'
0'
5'
c16t
c56t
cold competitor, 10 μM
kDa
-200-
-116-
-97-
-66-
-55-
-36-
-31-
reducing
non-reducing

FIGURE 2

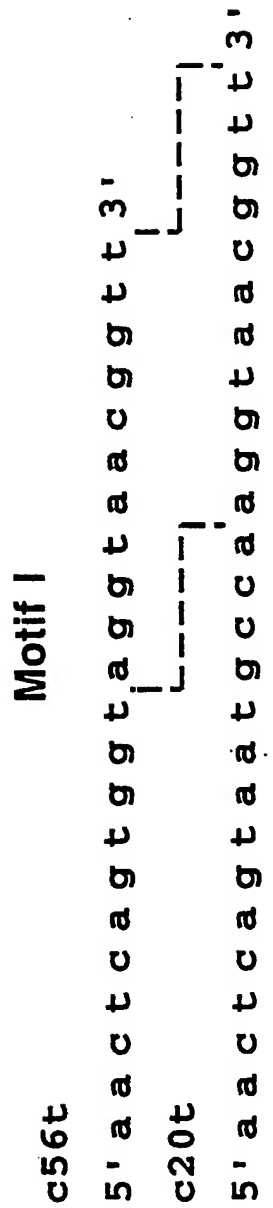

Motif I
c56t
5' a a c t c a g t g g t a g g t a a c g g t t 3'
c20t
5' a a c t c a g t a a t g c c a a g g t a a c g g t t 3'

FIGURE 3A

Motif II

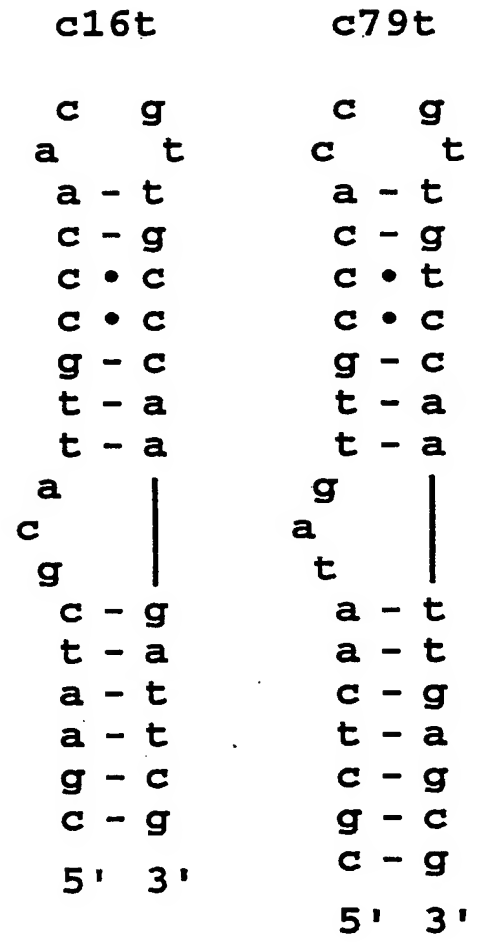
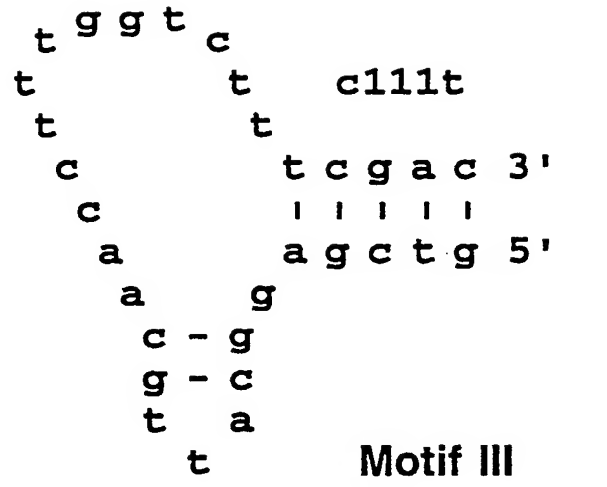


FIGURE 3B


t g g t c
t t c111t
t t
c t c g a c 3'
c | | | | |
a a g c t g 5'
a g
c - g
g - c
t a
t
Motif III

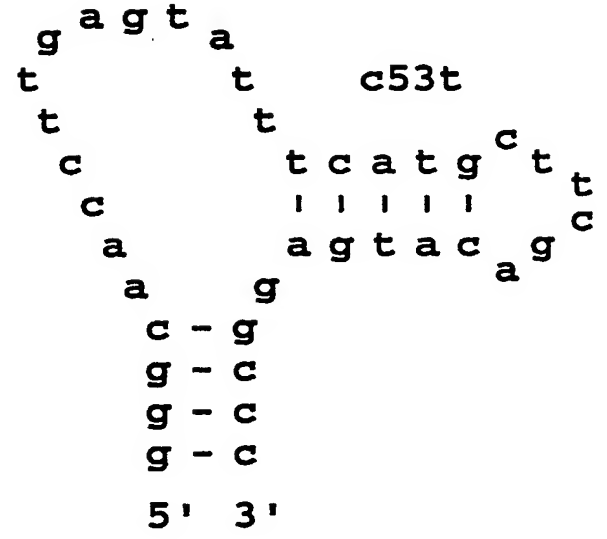

g a g t a
t t c53t
t t
c t c a t g c t t
c | | | | | c
a a g t a c g
a
a g
c - g
g - c
g - c
g - c
5' 3'

FIGURE 3C

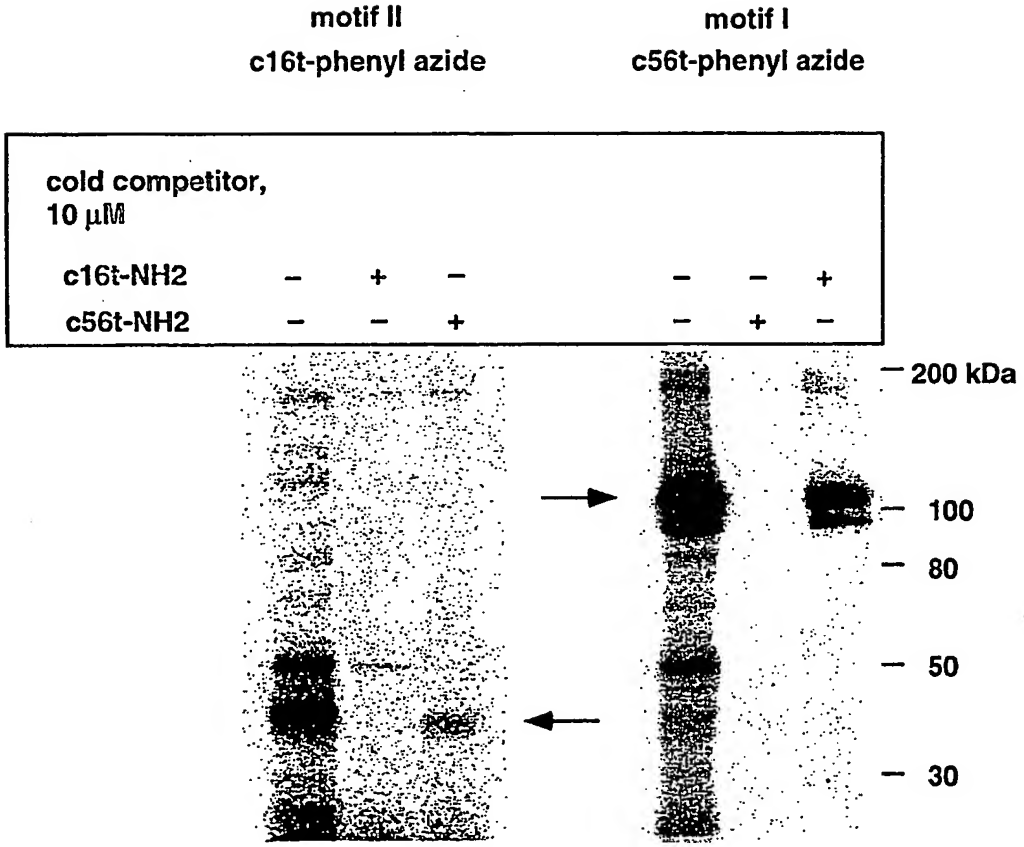

motif II
c16t-phenyl azide
motif I
c56t-phenyl azide
cold competitor,
10 μM
c16t-NH2 – + – – – +
c56t-NH2 – – + – + –
200 kDa
100
80
50
30

FIGURE 4

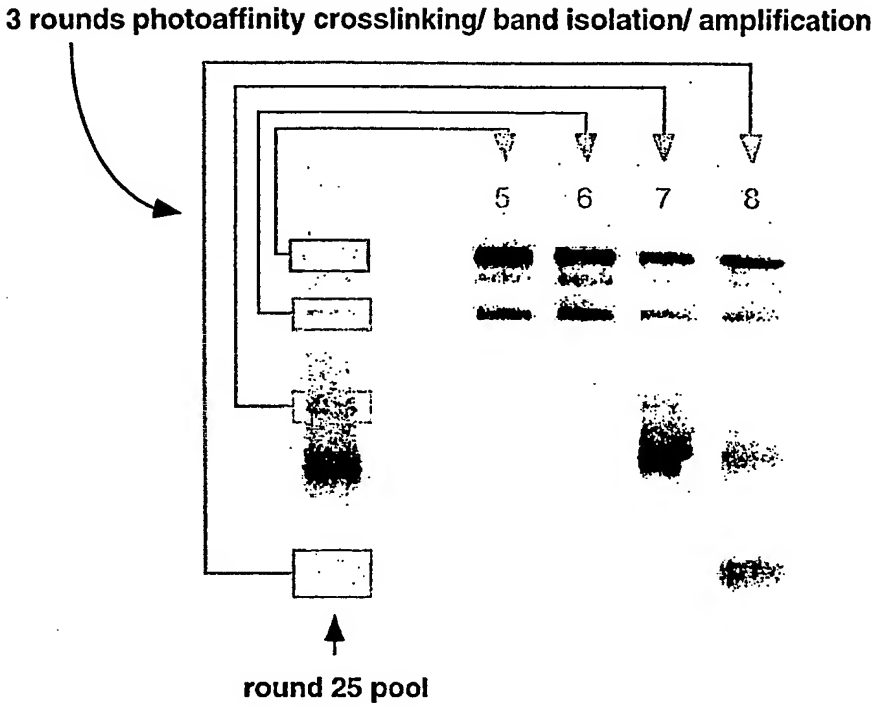

3 rounds photoaffinity crosslinking/ band isolation/ amplification
5
6
7
8
round 25 pool

FIGURE 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06060

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07H 21/02, 21/04; C12P 19/34; C12Q 1/68
US CL :435/6, 91.2; 536/22.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/22.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/14843 A1 (GILEAD SCIENCES, INC.) 03 SEPTEMBER 1992, PAGES 29-31, 94-102	1-30

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
25 JULY 1996

Date of mailing of the international search report
29 AUG 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
STEPHANIE W. ZITOMER, Ph.D.
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*